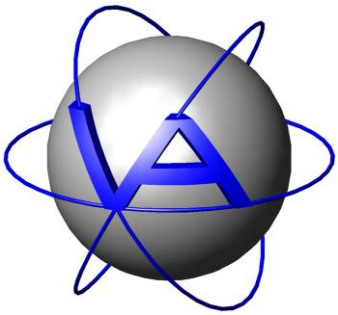

Activity Pattern

User's Manual

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Table of Contents

Foreword 15

Part One: User's Manual for Activity Pattern

1 Product Overview 17
1.1 Functions 17
1.2 Requirements 17
2 Installation of Activity Pattern 17
3 Start and main window 18
3.1 Loading files 19
3.2 The workspace 22
3.2.1 The data tab and the file menu 22
3.2.2 The charts tab and menu 24
3.2.3 Labeling a chart 25
3.2.4 Saving a chart 25
3.2.5 Deleting a chart 26
4 General functions 27
4.1.1 Settings 27
4.1.2 Selecting colours 29
4.1.3 Calendar function 29
5 Tools 29
5.1 Clear defaults 29
5.2 Manage connections 29
6 Actogram 31
6.1 Canvas 33
6.2 Axes 35
6.2.1 Bottom / Top 36
6.2.2 Left / Right 36
6.3 Palette 37
6.4 Background 39
6.5 Overlays 40
6.6 Legend 44

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7 Statistical chart by date 45
7.1 Data series 45
7.2 Canvas 48
7.3 Axes 48
7.3.1 Bottom 49
7.3.2 Left 50
7.4 Labels 51
7.5 Lines 52
7.6 Background 52
7.7 Legend 52
7.8 Log 53
8 Statistical by time 54
8.1 Data series 55
8.2 Canvas 55
8.3 Axes 56
8.4 Labels 56
8.5 Lines 56
8.6 Background 56
8.7 Legend 56
8.8 Log 56
9 Rhythm analysis 57
9.1 Data series 57
9.2 Canvas 59
9.3 Axes 59
9.4 Labels 60
9.5 Lines 60
9.6 Background 60
9.7 Legend 60
9.8 Log 60
10 Cosinor 61
10.1 Data series 62
10.2 Canvas 63
10.3 Axes 63

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10.4 Background 63
10.5 Legend 63
10.6 Log 64
11 Time Budget 65
11.1 Data series 65
11.2 Intervals 68
11.3 Axes 69
11.4 Labels 69
11.5 Lines 69
11.6 Background 69
11.7 Legend 69
11.8 Log 70
12 Appendix I: Keyboard shortcuts 71
13 Appendix II: Formats for date and time on actogram and chart axes 72
14 Appendix III: ADF Merge: Preparing data for ActivityPattern 73
14.1 Start of program 73
14.2 Cleaning data 74
14.3 Sort data 75
14.4 Delete data 75
14.5 Merge files 76

Part Two: Theoretical Introduction to Biological Rhythms and Rhythm Analysis

Chapter 1: Introduction 78
1 What is activity? 78
2 The ecological value of activity rhythms 79
3 Applied activity rhythm research – some ideas for the researcher 81
3.1 Activity Pattern research in the field 81
3.2 Habitat use – GPS plus activity sensor 82
3.3 Monitoring animal well-being 82
Chapter 2: A theoretical introduction into rhythm research 84
1 Biological rhythms 84
1.1 The properties of biological rhythms 84
1.2 Zeitgeber 86

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1.3 The relation between zeitgeber and endogenous rhythms ... 87
1.4 Masking ... 89
1.5 The multi-oscillator system ... 90
2 Plasticity of biological rhythms ... 91
3 An overview of activity rhythms ... 92
3.1 Daily or circadian rhythms ... 92
3.2 Seasonal or circannual rhythms ... 92
3.3 Ultradian and infradian rhythms ... 93
3.4 Tidal and lunar rhythms ... 94
4 Feedback loops ... 95
4.1 Neural control ... 96
4.2 Hormonal control ... 97
4.3 Genetic control ... 98
5 Disruptions and aberrations of rhythms ... 98
Chapter 3: Measuring activity ... 100
1 Characteristics of activity measurement by GPS Plus Collars ... 100
2 Basics of sampling intervals ... 101
3 The activity mode ... 103
4 Data gaps and interpolation ... 104
Chapter 4: Data analysis ... 106
1 Time series ... 106
2 Actogram or activity plot ... 106
3 Statistical charts ... 108
4 Diurnality Index ... 110
5 Peak Analysis ... 111
6 Single Cosinor ... 112
7 Rhythm analysis, Harmonic Part (HP) and Degree of Functional Coupling (DFC) ... 116
7.1 Aim of rhythm analysis ... 116
7.2 Mathematical background ... 117
7.2.1 Autocorrelation ... 118
7.2.2 Fourier transform ... 119
7.2.3 Basics on the duration of a time series ... 121
7.2.4 Successive Fisher-test ... 121

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7.2.5 Harmonic Part and Degree of Functional Coupling 122
Glossary 124
Further reading and references 127
Index 134

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Table of Figures

Figure 1: Main window 18
Figure 2: Window to load dataset from database 19
Figure 3: Details for database connection 20
Figure 4: Details for animal dataset 20
Figure 5: Dataset awaiting download 20
Figure 6: Dataset while being downloaded 21
Figure 7: List of available datasets 21
Figure 8: Workspace with loaded file 22
Figure 9: Window to unload a dataset 22
Figure 10: File menu 23
Figure 11: Opening the Charts menu from the Data tab 24
Figure 12: The Charts tab with an actogram 24
Figure 13: List of charts with title and description 25
Figure 14: Menu to edit the chart list 25
Figure 15: Window opened by right-click on the chart 25
Figure 16: Output options dialog 26
Figure 17: Window opened by right-click on the chart 27
Figure 18: Settings submenu 27
Figure 19: Lists of settings for defaults: top left: actogram settings, top right: statistical charts and rhythm analysis settings, bottom left: cosinor settings, bottom right: time budget settings 28
Figure 20: Reduced (left) and full (right) colour selection dialog with Define custom colours >> 29
Figure 21: Tool menu 29
Figure 22: Manage connections window 30
Figure 23: Input for connection details 30
Figure 24: Actogram settings 31
Figure 25: Actogram settings after selecting an ADF file 32
Figure 26: Histogram to select activity thresholds 33
Figure 27: Canvas section 33

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Figure 28: Labels section....34
Figure 29: Lines section....34
Figure 30: Axes section....35
Figure 31: Section for bottom and top axis....36
Figure 32: Section for left and right axis....36
Figure 33: Palette section....37
Figure 34: Palette creation dialog....37
Figure 35: Background section....39
Figure 36: Overlays section....40
Figure 37: Day grid dialog (left); actogram with day grid (right)....40
Figure 38: Hour grid dialog (left); actogram with hour grid (right)....41
Figure 39: Actogram with sun lines for sunrise, midday and sunset....41
Figure 40: Sun lines dialog (left) and selection box for parameter (right)....43
Figure 41: Sun lines dialog with Custom… selection....43
Figure 42: Legend section....44
Figure 43: Statistical charts by date. Left: For one dataset, mean activity (blue line) and median of activity (green line) is given. Right: For two datasets, average monthly activity plus confidence interval α = 0.05 is given. The y-axis gives the relative level of activity as measured by the collar....45
Figure 44: Data series section....45
Figure 45: Window for choosing the settings for Statistical chart settings. The same section is shown for Statistical… by time and Rhythm analysis....46
Figure 46: Data series section after one data series has been added....47
Figure 47: Canvas section....48
Figure 48: Axes section....48
Figure 49: Chart of mean monthly activity of two individuals. Left: default layout. Right: with margins 5 % of chart size....49
Figure 50: Bottom axis section....49
Figure 51: Left axis section....50
Figure 52: Labels section....51
Figure 53: Lines section....52
Figure 54: Legend section....52

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Figure 55: Log section 53

Figure 56: Statistical chart by time. Mean activity calculated in the course of the day for one month with an interval length (left) 5 minutes and (right) 60 minutes with confidence interval α = 0.05. 54

Figure 57: Settings window with list of ADF files for statistical chart by time 55

Figure 58: Chart with Harmonic Part (green) and Degree of Functional Coupling (blue), algorithms from Berger et al. (2003) and Sinz & Scheibe (1976). 57

Figure 59: Data series section for rhythm analysis 57

Figure 60: Window for choosing the settings for rhythm analysis 58

Figure 61: Log section 60

Figure 62: Cosinor-Plot for the 24 hour period. The blue line represents the vector of the cosinor with the direction showing the phase angle between the onset of the period and the acrophase (in this case equal to the time of day, for annual cosinor equal to the date and time of the year), its length indicates the amplitude; the circle (in some cases an ellipse) indicates the confidence interval. If the radius of the confidence interval is greater than the amplitude, the calculated rhythm is not significant for the selected p-value. 61

Figure 63: Data series section for cosinor analysis 62

Figure 64: Window for choosing the settings for cosinor analysis 62

Figure 65: Log section for cosinor, tested period 24 hours 64

Figure 66: Charts for Time Budget. Left: Total daily activity time (left y-axis) and mean duration of single activity peak (right y-axis). Right: Diurnality index, positive values indicate relatively more activity at daytime, negative values indicate relatively 65

Figure 67: Data series section for time budget 65

Figure 68: Window for choosing the settings for time budget 66

Figure 69: Intervals section 68

Figure 70: Drop-down menu for defining intervals 68

Figure 71: List of Intervals (example). Interval Dawn starts with the start of nautical twilight and ends with sunrise, when interval Day starts. 69

Figure 72: Log section. The detailed numerical results of the time budget analysis can be accessed in the log section. Results are even given calculated for every single day or for the complete data range. This list includes the following parameters (In this example, the default intervals *Day* (interval 1) and *Night* (interval 2) are given.

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The terms *Day* and *Night* will change if you rename the intervals with the *Interval* section): 70

Figure 73: ADF Merge start window 73

Figure 74: Dataset with sets of continuous data (left) and list of data points with highlighted defects (right) 74

Figure 75: Go to menu 75

Figure 76: Edit menu 75

Figure 77: List of overlapping time periods in two ADF files to be merged 76

Figure 78: Activity level (grey bars) and distance (red line) between two GPS positionings for a female roe deer over 24 hours measured in 15 minute intervals (measured by the GPS Plus Collar) 78

Figure 79: Diagram of an oscillation 84

Figure 80: Example for a free-running rhythm in a diurnal animal. Yellow = light, α = time of activity, red = rhythm with regular onset of activity, black = free-running rhythm. 85

Figure 81: Path of the zeitgeber light 87

Figure 82: Advanced and delayed phase angles. Yellow = zeitgeber signal light, red = activity 87

Figure 83: Example for transients in a diurnal animal. Yellow = light, □ = time of activity, red = steady-state oscillation prior to shift, black = transients immediately after shift, blue = steady-state oscillation after shift. 88

Figure 84: Two simple examples of periodic masking. Left: Activity is immediately induced by the masking agent (e.g. social partner) but returns instantly to the phase angle with the zeitgeber when the masking agent is removed. In contrast to a zeitgeber, there are no transients between both states. Right: Activity during the physiological activity time is suppressed while the masking agent (e.g. predator) is present and resumes after it is removed. The original rhythm is displayed when the masking agent does not appear anymore. 89

Figure 85: Schema of a multi-oscillator system. The pacemaker is superior to the other oscillators and enforces its oscillation on them. However, due to their coupling with each other, the inferior oscillators influence the pacemaker too, but their influence is weaker. On the second level, the oscillators are coupled to and therefore influence each other. 90

Figure 86: Schema of a feedback loop 95

Figure 87: Interaction between endogenously controlled circannual, circadian and twilight-rhythms and the ultradian rhythms as the Additive Model after Scheibe et al.

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(2001). The circannual (1), the circadian (2), the twilight (3) and the ultradian oscillations (4) are added to a resulting daily feeding pattern (5), which is similar to an observed feeding pattern measured on Przewalski horse in a semireserve (5). 96

Figure 88: Schema of a mammal brain (median-sagittal) 97

Figure 89: The three axes used for activity measurement by the GPS Plus Collar (Photo: BPCP, Botswana). 100

Figure 90: Time series of activity sampled in different intervals. It is obvious that the pattern of activity is not detectable with a long sampling interval and the measured pattern changes with the time of sampling. The smaller the sampling interval, the more precise is the information. 102

Figure 91: Angle between collar position and vertical axis (Photo: Bianka Schirrmann) 103

Figure 92: Actogram over eight days. For every sampling interval (in this case every five minutes, resulting in 288 data points per day) the level of activity is indicated by different colours. 106

Figure 93: Actogram over about five months for a mostly nocturnal animal. Curved lines indicate sunrise and sunset. 107

Figure 94: Double (left) and triple (right) plot actogram of the same dataset as in Figure 88. Curved lines indicate sunrise and sunset. The nocturnal pattern is fully plotted and transients over night are visible. 107

Figure 95: Zoom into double plot actogram over six days. 108

Figure 96: Statistical charts with different sampling interval showing mean values. (A) Statistical by date, blue: sampling interval one day, red: sampling interval one month. The peaks in early August appear only weakly in the monthly sample curve, because of low values in the second half of the month; the peaks in June are leveled out by lower activity values in the same month. (B) Statistical by time, blue: sampling interval 5 min, red: sampling interval 60 min. Short peaks and nadirs disappear. 109

Figure 97: Actogram and associated mean chart by time. While the big peaks are related to a daily period of activity, this is not the case for the medium peaks. In the actogram transients (red lines) are visible which are lost in the mean activity chart. 110

Figure 98: Exemplary time series with peaks counted for activity threshold 10 (red lines) and activity threshold 30 (green lines). Minimum peak length in both cases 10 minutes. 112

Figure 99: Estimations of acrophase (blue) and amplitude (red) for a period length of 24 hours or 365 days by cosinor calculation 113

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Figure 100: Graphical presentation of Cosinor results. The red line resembles the vector of the Cosinor with the direction showing acrophase (blue), its length indicates the amplitude; the green circle (in some cases an ellipse) indicates the confidence interval. If the radius of confidence interval is bigger than the amplitude, the calculated rhythm is not significant for the selected p-value. 113

Figure 101: Cosinor calculation for the body mass of Przewalski horses (after Scheibe and Streich 2003,courtesy of authors). A cosine function (black line) with the period length 365 days is fitted in phase and amplitude to the measured time series (green crosses). The acrophase of 282.3 equals the 13th of October, the amplitude was 22.4 kg. The result is significant as indicated by the red circle which is smaller than the amplitude (green vector). 114

Figure 102: Examples for Single Cosinor results for time series with two maxima. Acrophase differs with the relative size of each maximum and the distribution of the remaining activity. 115

Figure 103: Original time series (period length = 10 days), its autocorrelation and its power spectrum during the time series analysis procedure. 117

Figure 104: Calculation of autocorrelation for a time series with sampling interval 15 minutes. The original time series (black) (a) is duplicated and correlated with itself (green); the correlation is 100% (b). The duplicated time series is shifted for one sampling interval and again correlated to the original; the correlation is 98.5% (c). Shifting is repeated (d) until only half of the time series are overlapping (e). The autocorrelation coefficients are plotted against the number of sampling intervals for the shift (f)..... 118

Figure 105: Fourier transform as performed by Activity Pattern. A cosine function which period length equals the length of the autocorrelation function is fitted to the autocorrelation function and its amplitude and power is calculated (a). Then, a cosine function with the period lengths of half the lengths of the autocorrelation function is fitted (b). In the next steps, cosine functions with the period lengths of a third, fourth, fifth, etc. of the autocorrelation function are fitted (c,d). The calculated power of each significant period can be plotted against the period length in a power spectrum (e). 120

Figure 106: Example for the calculation of Harmonic Part and Degree of Functional Coupling. The Harmonic Part is the sum of the power of all significant harmonic periods. The Degree of Functional Coupling is the relation of the power of all significant harmonic periods and the power all significant (harmonic and non-harmonic) periods. 123

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Foreword

This manual is divided in two parts. Part I is a user's manual to the features of the program Activity Pattern. Part II will give you a basic introduction into the study of activity data, biological rhythms and the theory of all analysis tools used in Activity Pattern. It also provides information on how activity is measured and stored in the GPS Plus Collar. We suggest to start reading Part II before you configure your collars to choose the activity measurement, sampling interval and activity parameter most suitable for your study.

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Part One:

User's Manual for

Activity Pattern

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This chapter will guide you through the program Activity Pattern. For deciding which analysis you might use and data interpretation please refer to Part II, Chapters 3 and 4.

1 Product Overview

1.1 Functions

Activity Pattern is used to analyse activity data obtained from the GPS Plus collars. Its functions are:

- Plotting activity
- Calculating and plotting mean activity, median of activity including confidence interval and standard deviation
- Total time active per given period of time, day-night relation of activity, number and duration of activity peaks
- Rhythm analysis including autocorrelation, Fourier analysis, Harmonic Part and Degree of Functional Coupling
- Single Cosinor calculation

All results can be exported as text or as graph.

Note: You can only analyse and plot activity data from ADF files, but not edit ADF file. To do this, use the tool ADF Merge and follow the instructions given in Appendix III.

1.2 Requirements

Activity Pattern runs on

- Windows 7
- Windows Vista
- Windows XP

2 Installation of Activity Pattern

The installation of Activity Pattern is easy and straight forward. Use the file ActivityPattern_1.2_Setup.exe and follow the instructions. During installation, you have the possibility to associate all ADF files (activity data files) and VAP files (Activity Pattern workspaces) to Activity Pattern.

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3 Start and main window

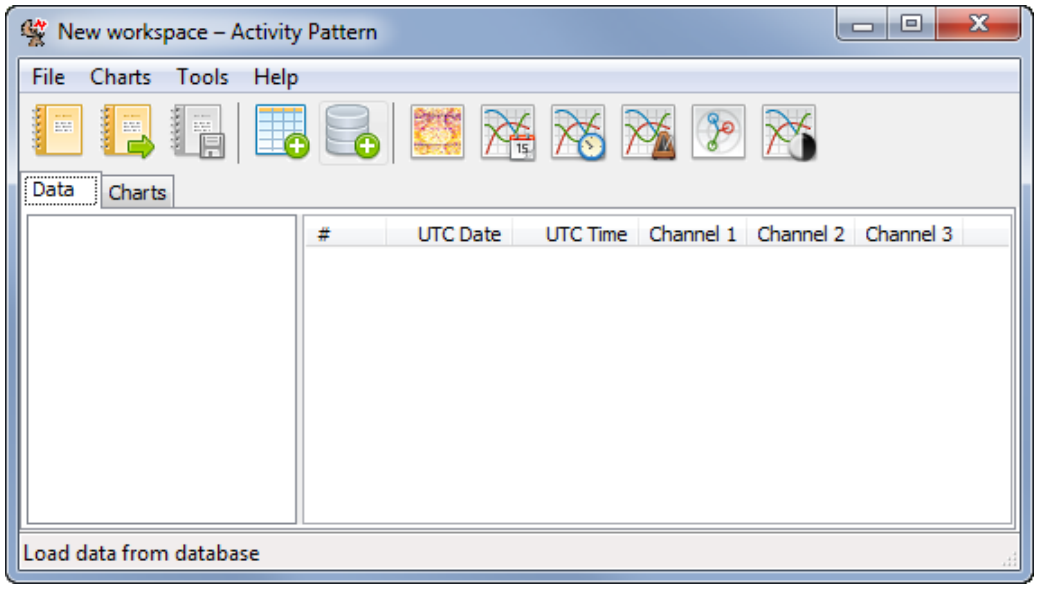

Figure 1: Main window

The workspace consists of two tabs to work in, the *Data* tab and the *Charts* tab. In the *Data* tab, all loaded ADF files will be shown in the left window and the data of a selected file will be shown in the right window. In the *Charts* tab, the charts will appear automatically after you created them (see 3.2.2).

There is also a list of icons as shortcuts to several functions:

creates a new workspace; you can also use the shortcut [Ctrl +N]

opens an existing workspace; you can also use the shortcut [Ctrl +O]

saves the openend workspace; you can also use the shortcut [Ctrl +S]

loads an ADF file from any folder of your computer; you can also use the shortcut Ctrl +L

loads data from your GPS Plus database; you can also use the shortcut [Ctrl +D]

creates an actogram (see 6 and Part II, chapter 4.2); you can also use the shortcut [Ctrl +1]

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creates a statistical chart by date (see 7 and Part II, chapter 4.3); you can also use the shortcut [Ctrl +2]

creates a statistical chart by time (see 7 and Part II, chapter 4.3); you can also use the shortcut [Ctrl +3]

performs a rhythm analyses and creates a chart (see 9 and Part II, chapter 4.7); you can also use the shortcut [Ctrl +4]

performs a cosinor analyses and creates a chart (see 10 and Part II, chapter 4.6); you can also use the shortcut [Ctrl +5]

calculates activity time, peaks and/or diurnality index and and creates a chart (see 64and Part II, chapters 4.4 and 4.5); you can also use the shortcut [Ctrl +6]

3.1 Loading files

The first step in Activity Pattern is to load an ADF file into the program. There are two sources for data. ADF files stored as single files on your computer or your network can be loaded through the *File* menu with *Load ADF file…* (Figure 10) *or* *Ctrl + L*.

If you use the GPS Plus Database, you can also load datasets directly from the database with *Load from database or* *Ctrl* + D.

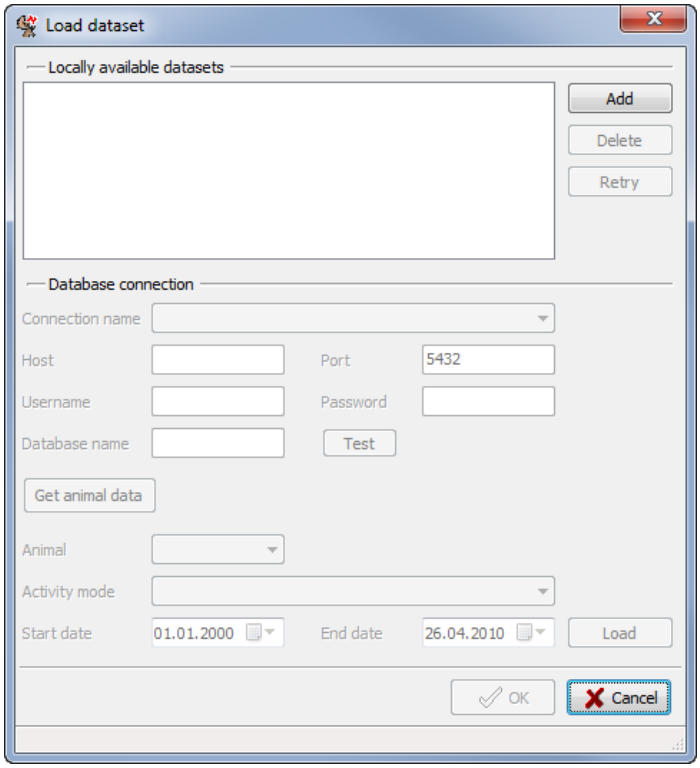

Figure 2: Window to load dataset from database

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To load a dataset from the database, first press Add. Then you can enter the details for the database connection. Type in the host, username, password and database name. The standard port number for the GPS Plus database is preset, but can be changed manually if needed. You can also define database connections which you can choose from the drop-down list. This is done using *Tools* ⇨ Manage connections *(5.2). If you type in the details, you can test if a connection can be established.*

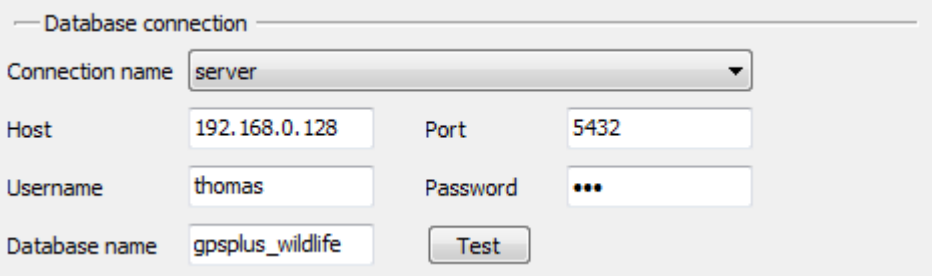

Figure 3: Details for database connection

When your database connection is established, press Get animal data. Choose an animal from the drop down list. Animals can be identified by their name and a five-digit ID number which has been assigned when adding the individual to the database. The database entries for these animals will be automatically checked for the used activity modes; choose one mode. Now the date range for the combination of the chosen animal and activity mode is checked. Press Load to transfer the dataset in the list of locally available datasets.

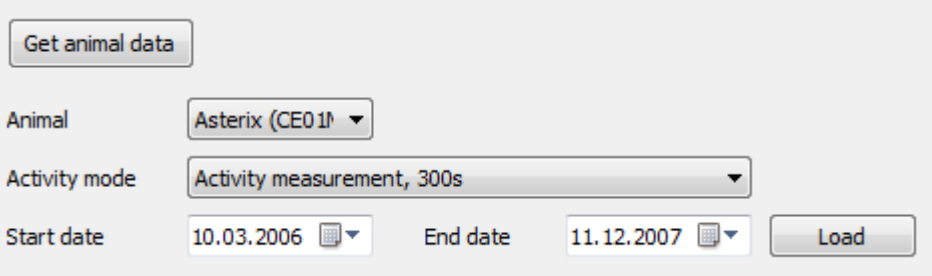

Figure 4: Details for animal dataset

The dataset will appear with information of animal ID, observation time, activity mode, source database and access date. At this stage it will be marked with a green plus sign.

Figure 5: Dataset awaiting download

The dataset will not be downloaded to your computer until you press OK. Then the database window will close and the dataset will appear in the left section of the data tab with the note

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Loading... . If you reopen *Load from database...* while the dataset is downloaded, it is marked with a green arrow. During the download, you can work with other datasets already loaded into your workspace.

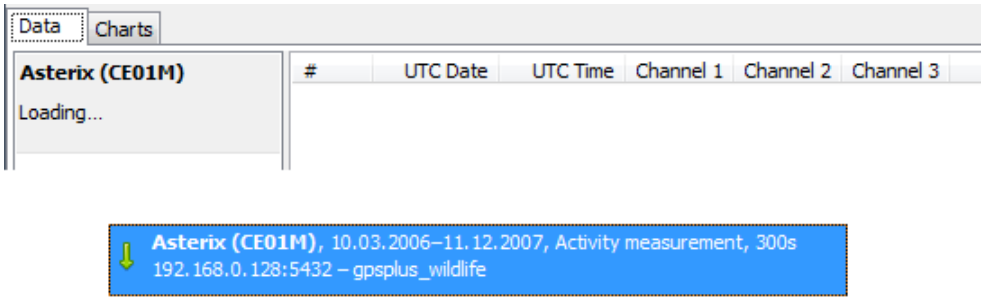

Figure 6: Dataset while being downloaded

After the download the datasets are stored on your computer and can be accessed without database connection. Changes done in the database (e.g. adding of new data) have no influence on your dataset and it is not possible to update the local dataset. If you want to add new data for an animal, you need to download a new dataset and redo the analysis.

If you want to download a dataset but not include it to your workspace at present, use Ctrl + left click. Its colour will change from blue to grey. When you press OK the dataset will be downloaded and kept in store, so you can load it into your workspace at a later time.

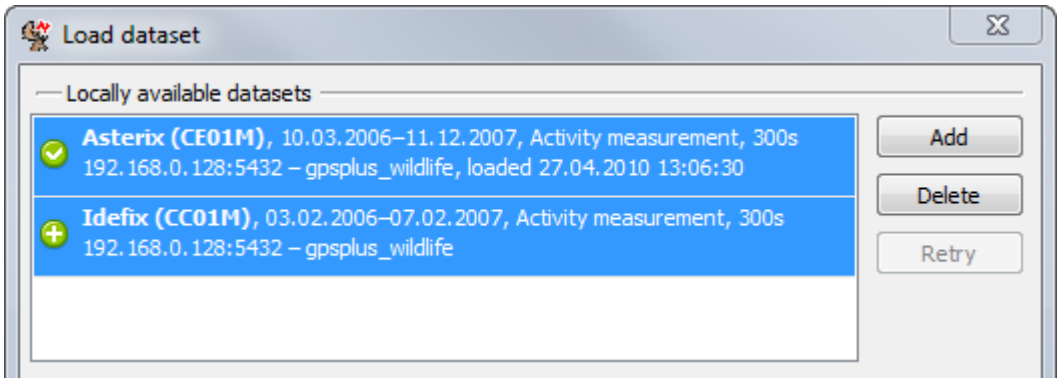

Figure 7: List of available datasets

After the download, datasets are marked with a green check mark. All datasets which are downloaded or waiting to be downloaded are listed as *Locally available datasets*. Press Delete to delete a dataset from your computer. It cannot be restored but must be downloaded from the database again.

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3.2 The workspace

3.2.1 The data tab and the file menu

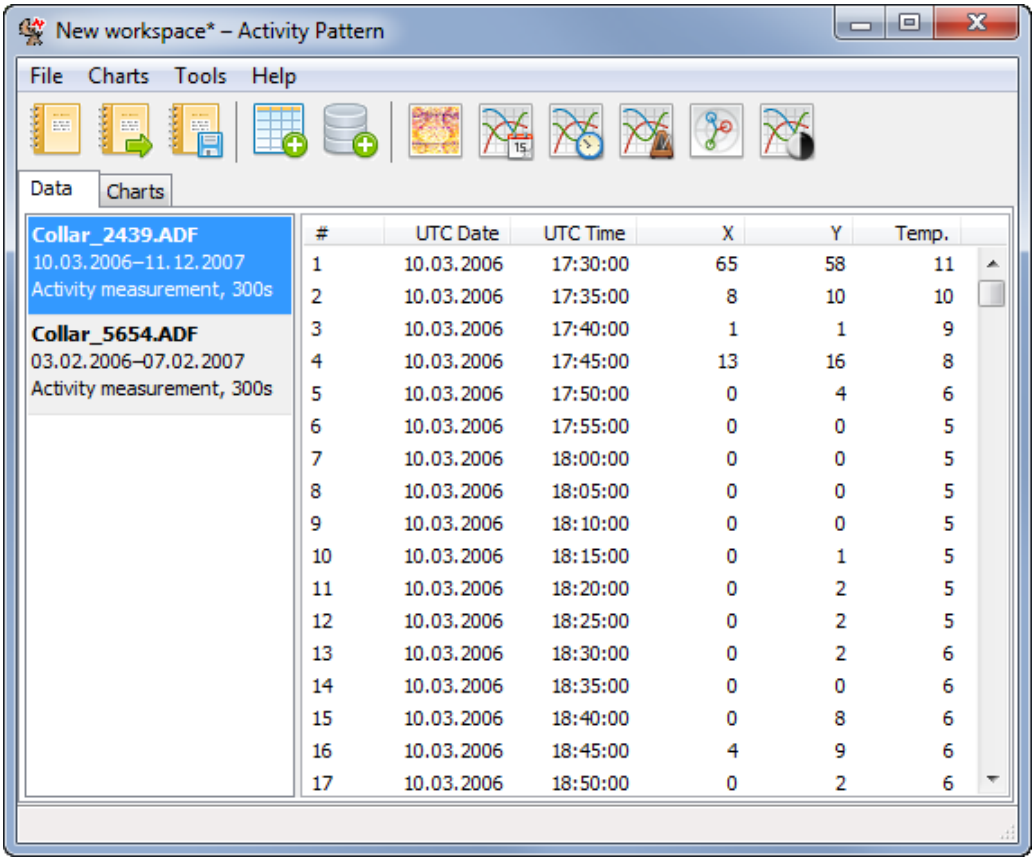

Figure 8: Workspace with loaded file

The loaded ADF files are listed in the left section of the data tab. For each file the file name, the data range and the name of the activity mode is given. To display the content of an ADF file in the right window, select its name with the mouse. The columns show the running number of data points (#), the UTC date and the UTC time. Columns 4 and 5 display the acceleration data depending on the mode you have chosen for activity sampling (e.g. X and Y). *Temp*. displays the temperature measured by the collar. You can highlight certain data points with the cursor, but you cannot edit the data.

To unload an ADF file, click on it with the right mouse button and select *Unload dataset*. Datasets downloaded from the GPS Plus Database will be removed from your computer and have to be downloaded again if you want to analyse them.

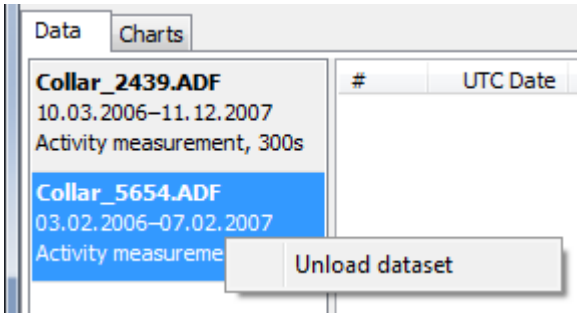

Figure 9: Window to unload a dataset

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If any charts are associated with the dataset you want to unload, the following message will appear: "There are charts using *"[FILENAME]" as their data basis. Please delete them first.*"

To organise your workspace use the file menu.

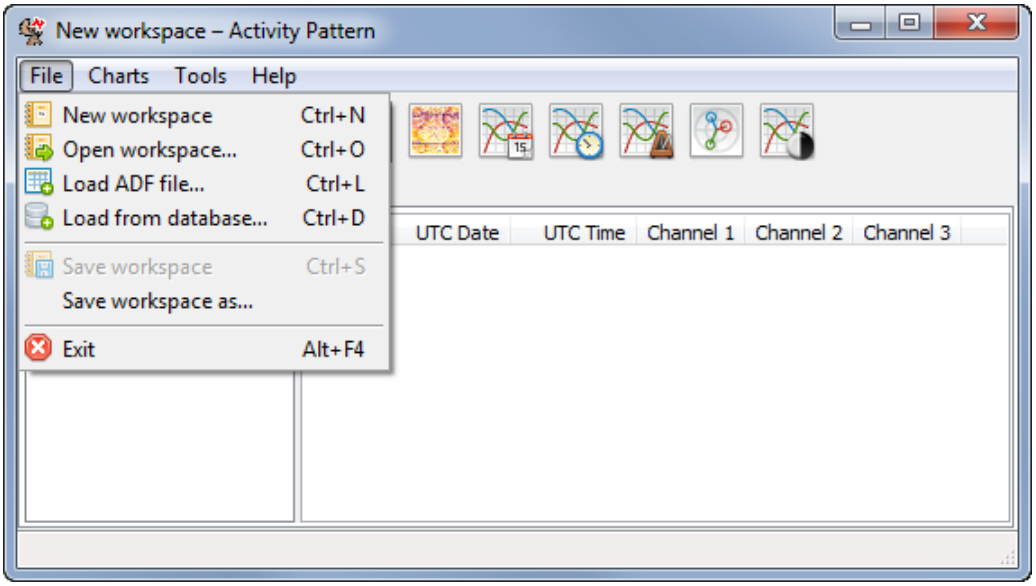

Figure 10: File menu

To save your workspace to disk use *File → Save workspace as…* . If your workspace includes ADF files which are not associated with a chart, the following message will appear: "*Dataset "[FILENAME]" has no charts associated with it. Do you wish to exclude it from saving?*" This option will reduce the size of the workspace file. Press *No*, if you want to save the file to the workspace.

To create a new workspace use *File → New workspace* or Ctrl + N. To open an existing workspace use *File → Open workspace …* or Ctrl + O.

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3.2.2 The charts tab and menu

All analyses available in Activity Pattern can be initiated through the *Charts* menu.

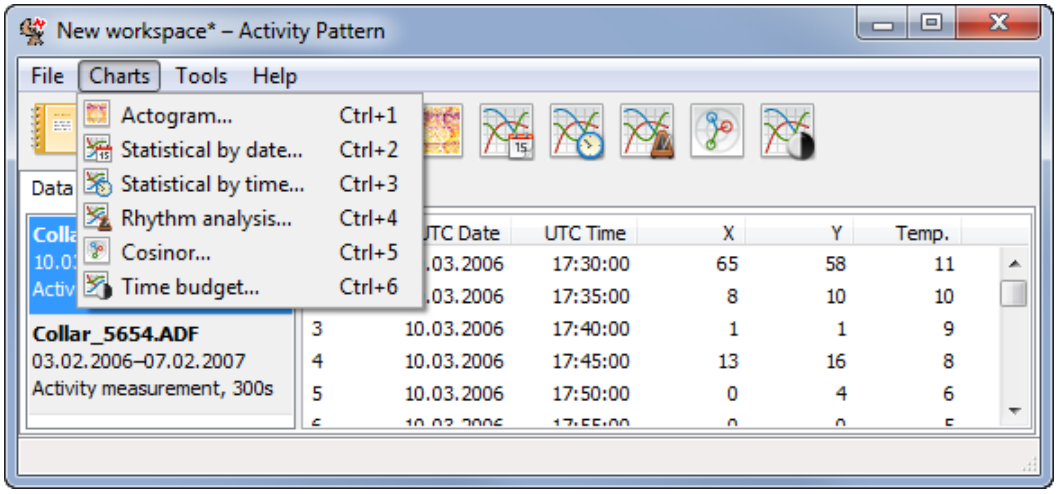

Figure 11: Opening the Charts menu from the Data tab

After a chart has been created, the focus will automatically switch to the *Charts* tab. On the left, a list of all charts is given, on the right the charts themselves are displayed.

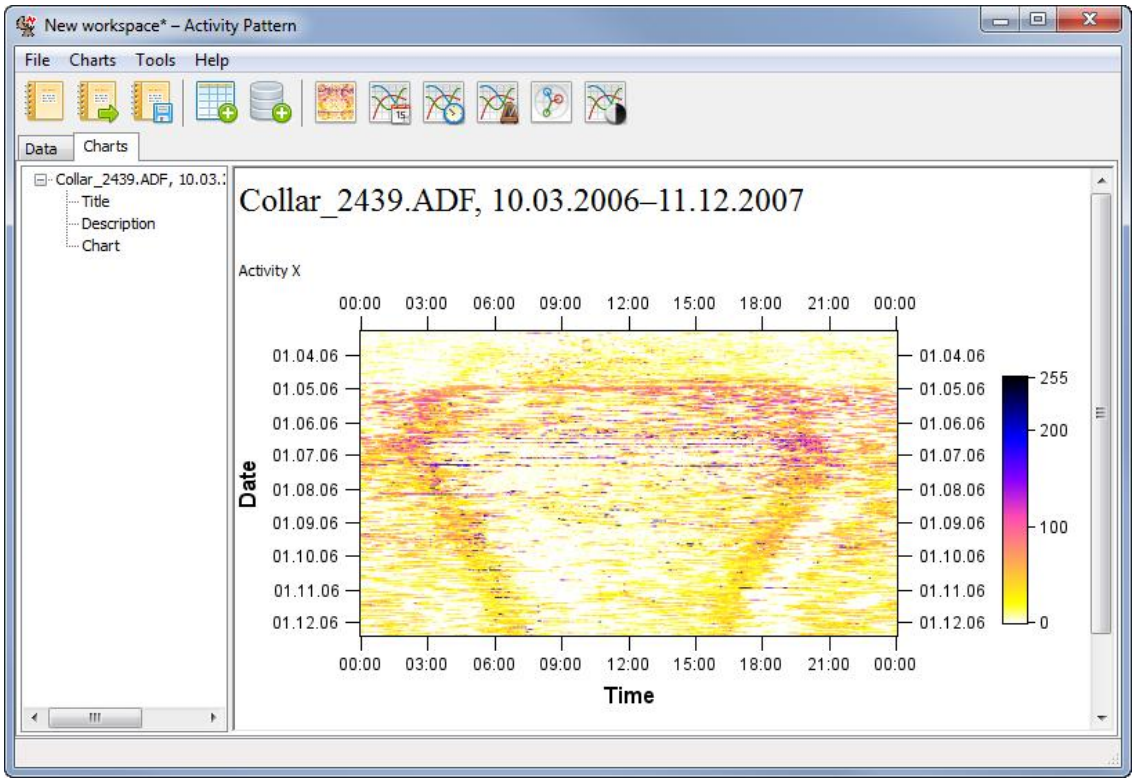

Figure 12: The Charts tab with an actogram

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3.2.3 Labeling a chart

When you create a chart, it will automatically be given a title and will be listed in the left section of the charts tab. In case of an actogram, this title consists of the name of the plotted file and the selected time period. For all other charts, the title is constructed from the analysed time period and the chart's type.

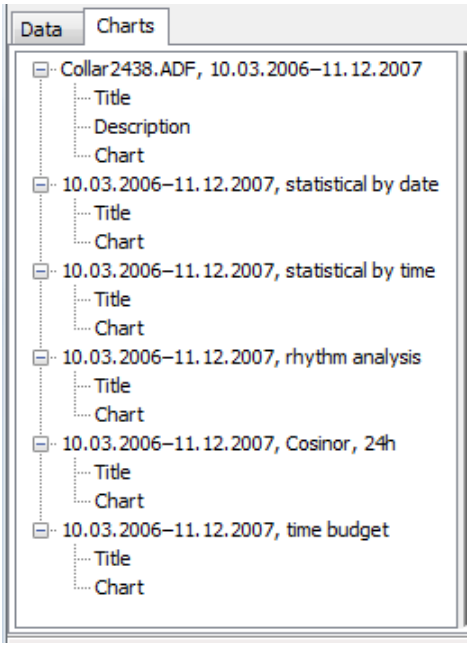

Figure 13: List of charts with title and description

To change the title, double-click it in the right part of the window, above the chart. A window will open where you can edit the text. To erase the title, simply delete the text. Thereafter, a new title can be added by right-clicking [Chart 1] and selecting *Add title* from the context menu (Figure 14).

Figure 14: Menu to edit the chart list

Adding, editing, or removing a description is done in a similar fashion. If a chart does not have a description so far, you can add one by right-clicking the corresponding entry in the left part of the window and choosing *Add description* from the context dialog. To edit a description, double-click it. Erasing the text will delete the description.

3.2.4 Saving a chart

To export a chart, right-click on it and choose the option *Save graphic as…* (Figure 15) to open the *Output options* dialog (Figure 16).

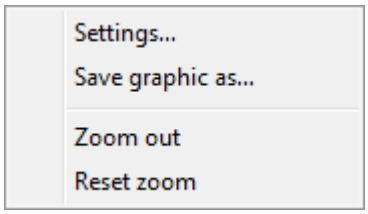

Figure 15: Window opened by right-click on the chart

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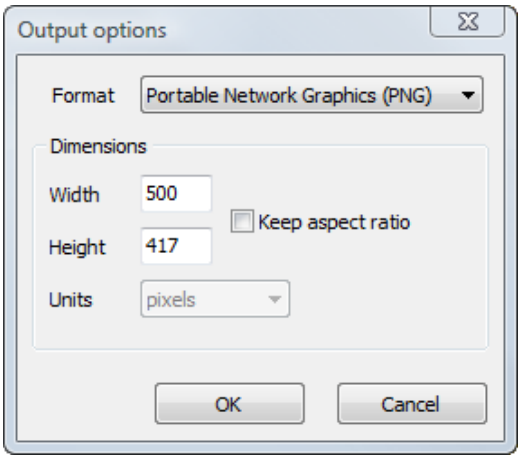

Figure 16: Output options dialog

The *Format* field allows you to choose between Portable Network Graphics (PNG) or Encapsulated PostScript (EPS).

PNG	bitmap graphic suitable for beamer presentations
EPS	vector graphic which can be edited with a vector graphic program (e.g. Adobe Illustrator); bigger in size, but suitable for publication

With *Dimensions*, you can choose the size of the graphic. Dimensions can be given in millimeter, centimeter, points and inches. Check the *Keep aspect ratio* box to keep the size relations in the chart.

3.2.5 Deleting a chart

To delete a chart, right-click on the name of the chart in the charts tab and select *Delete chart (*Figure 15*).*

Note: There is no possibility to undo the deletion.

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4 General functions

There are some functions which are accessible from various sections of the program.

4.1.1 Settings

To access the chart settings dialog, either double-click on the actogram, or press the right mouse button and select *Settings*. After performing the desired changes, you can redraw the chart by clicking Apply to keep the dialog open, or OK, closing the dialog. Additionally, changes to some parameters will be reflected immediately.

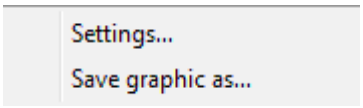

Figure 17: Window opened by right-click on the chart

A chart's configuration (e.g. its palette or the layout of the axes) can be transferred between charts by exporting it to a file and afterwards importing into another chart. To do this press the *Template…* button in the settings dialog. *Export…* will save the current configuration as a file. *Import…* will load a previously saved configuration. *Set as default* lets you define the current settings as default for charts of the same type that will be created from then on.

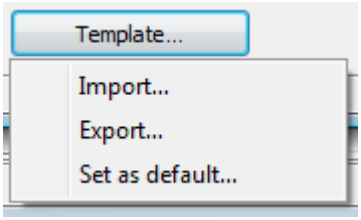

Figure 18: Settings submenu

If you want to export your settings or set them as default, a new window will open to allow you a choice between different parameters. By checking and unchecking the box you can decide which parameters you want to include in the file. The settings will be saved as CFG file in a folder of your choice. You can only save settings related to the chart's layout, coordinates or time corrections need to be typed in for every new actogram.

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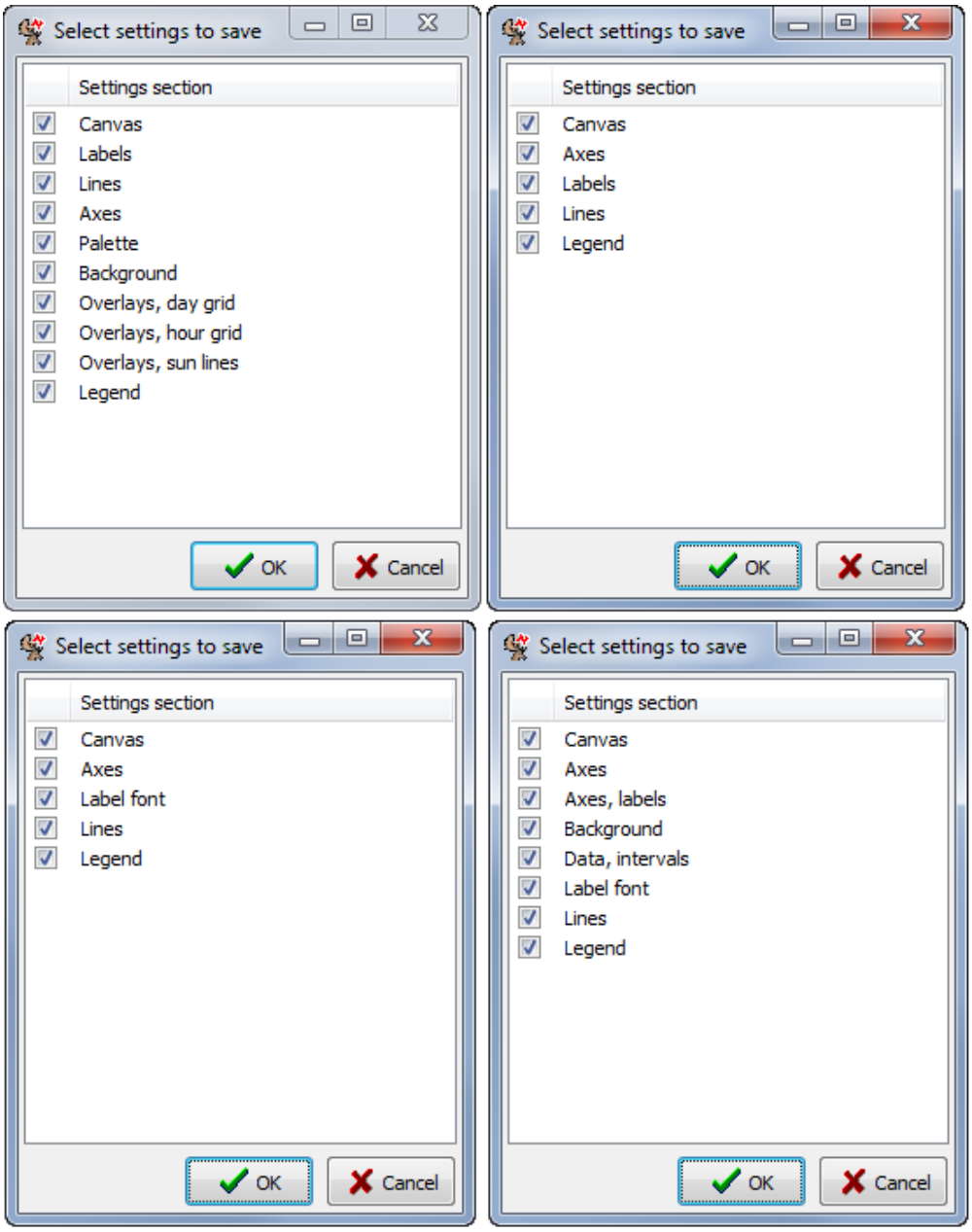

Figure 19: Lists of settings for defaults: top left: actogram settings, top right: statistical charts and rhythm analysis settings, bottom left: cosinor settings, bottom right: time budget settings

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4.1.2 Selecting colours

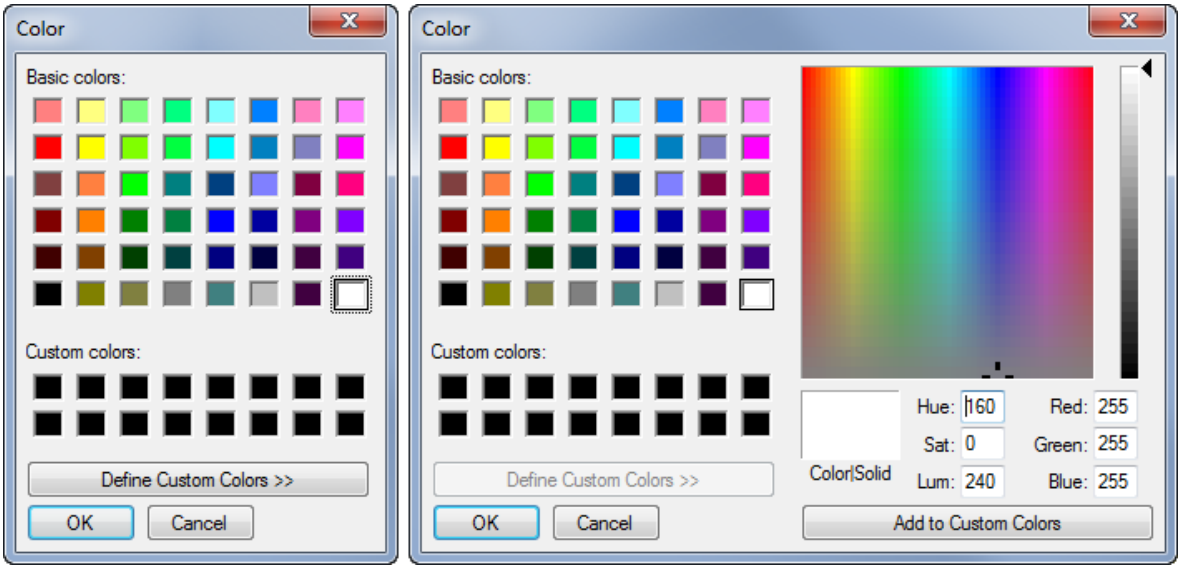

Figure 20: Reduced (left) and full (right) colour selection dialog with Define custom colours >>

Colours in the charts can be changed with the *Color* dialog. It can usually be accessed by double-clicking a colour box in the setting. You can choose between 48 basic colours or define them with Define custom colours >>.

4.1.3 Calendar function

To choose a date, either type in the required date or use the calendar function by clicking on the drop-down arrow to the right of the date. This will open the visual date selection dialog of MS Windows.

5 Tools

5.1 Clear defaults

In some cases it might be necessary to erase the default settings due to severe problems with the configuration. In this case select *Tools* ⇨ *Clear defaults*.

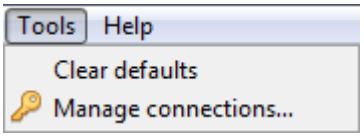

Figure 21: Tool menu

Note: Be careful to use this command, because all your personal settings will be erased and cannot be restored.

5.2 Manage connections

If your regularly download data from the database it will be useful to preset database connections. This can be done with *Tools* ⇨ Manage connections.

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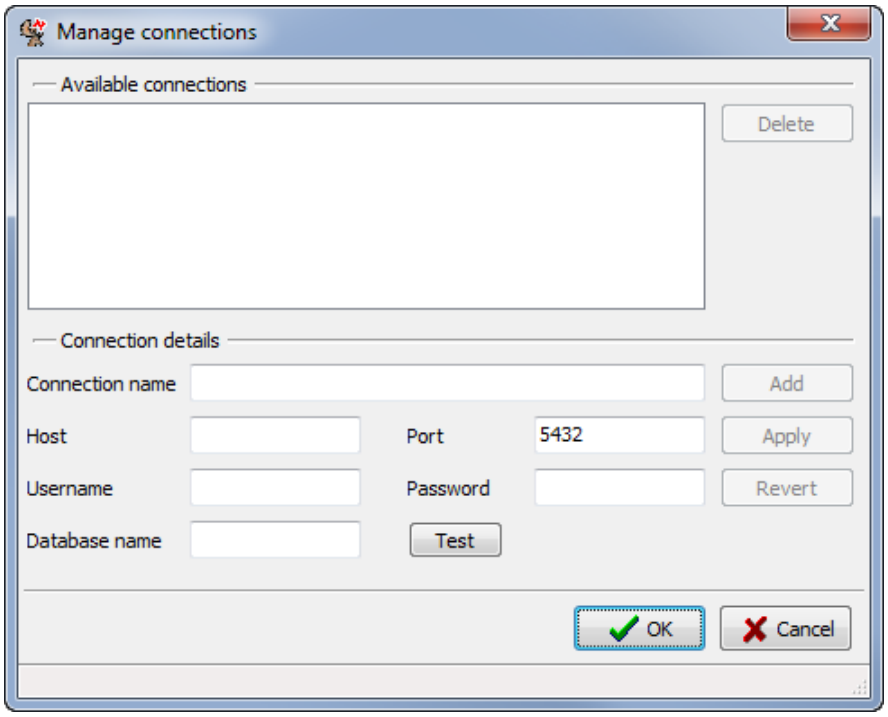

Figure 22: Manage connections window

Start by typing the connection name, host, username, password and database name.

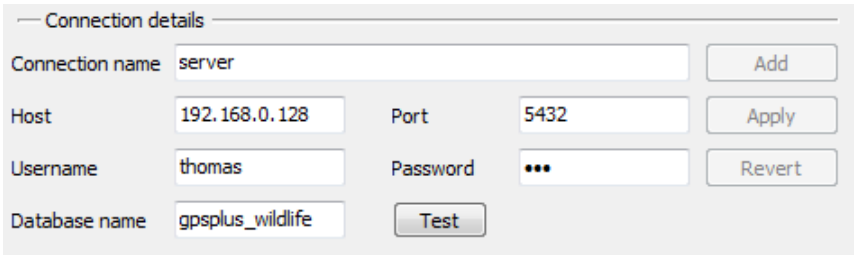

Figure 23: Input for connection details

Press Add to add the new connection to the list of available connections. Use Test to test if all details have been added correctly and the connection is running, but even without the manual test the connection will be tested before it is added to the list. If no connection can be established, the message "*No connection could be established with these parameters. Do you wish to use them anyway?*" will appear. This might be caused by false details, but can also happen if your database server is offline. Choose Yes to keep the details and try to establish the connections later, or choose No to change the details.

If you want to change the details of an existing connection, choose it from the list, make your changes and press Apply. If you want to undo changes before you have added or applied them to the list, use Revert.

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After setting up the *database* connection, you can upload datasets with Load from database or Ctrl + D.

6 Actogram

The actogram is a detailed plot of activity, displaying every single data point within a selected time span (for explanation see Part II, Chapter 4-2). Selecting *Charts* ⇨ *Actogram* from the main menu or pressing Ctrl + 1 will open the actogram settings dialog (Figure 24). Sections with missing compulsory input are indicated with an asterisk.

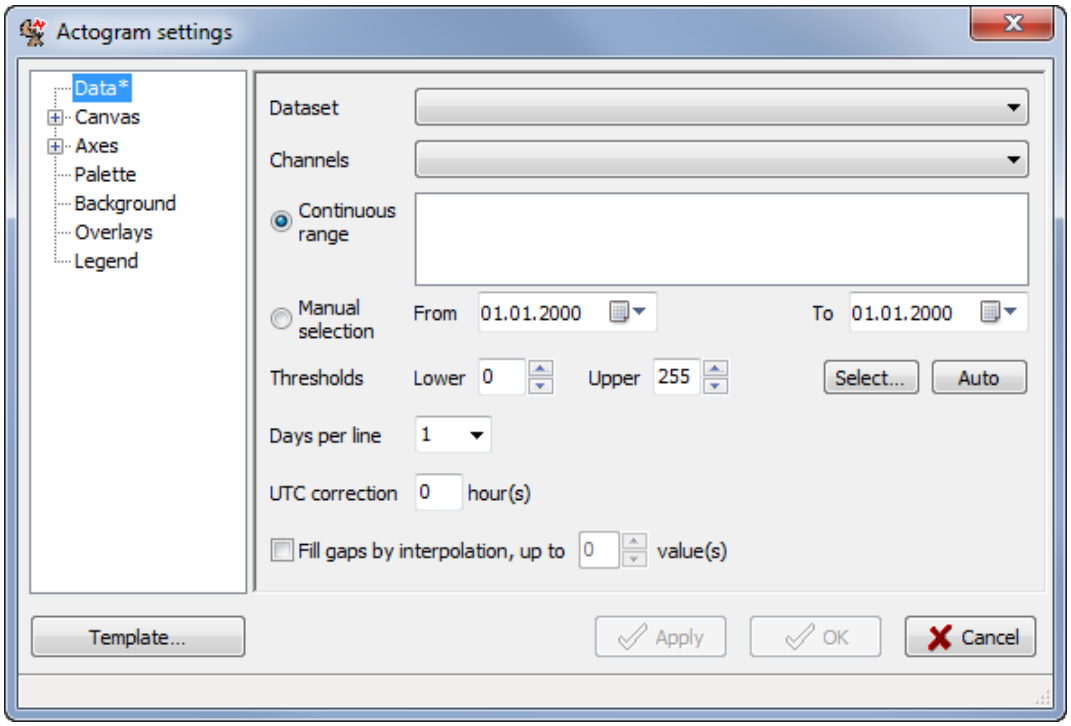

Figure 24: Actogram settings

To create an actogram, but remain in the settings dialog, press Apply. OK creates an actogram and closes the dialog. Choose the data and the channel you want to plot with *Dataset* and *Channel* The default setting is the first channel of the ADF file, e.g. Activity X for modes 1-4, (Figure 25).

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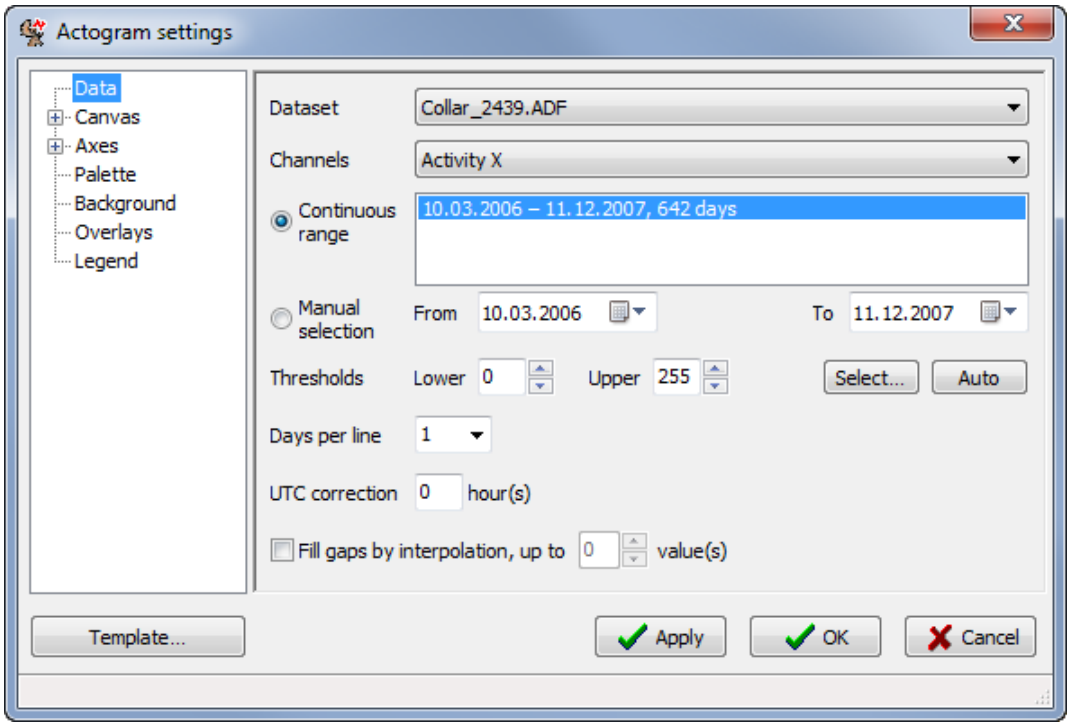

Figure 25: Actogram settings after selecting an ADF file

After selecting an ADF file, the date range in which data is available is shown in *Continuous range*. Data is organised in one block until a data gap of 60 minutes occurs. Then a new block begins. The longest block of consecutive data is selected automatically, but you can select another range instead. Alternatively, you can select all ranges at once by double-clicking the range list, or any time range with *Manual selection*.

By default, all activity levels between 0 and 255 are displayed (for detailed information on activity measurement and storage see Part II, Chapter 3). If you want to focus on certain activity levels, you can define an upper and lower *Threshold*. To help you with the selection, the button Select... opens a dialog with a histogram (Figure 26). You can either use the mouse to select the thresholds by clicking the histogram and dragging the selection frame while holding the left mouse button, or type the thresholds in directly into the text fields next to *Selection*. The boxes on the right side of the window show how much of the data lies inside, below and above the selected thresholds.

The button Auto sets the lowest and highest activity value within the dataset as thresholds.

With *Days per line* it is possible to display more than one day per line. You can choose between one, two or three consecutive days (see Part II, Chapter 4-2, Figure 92 till Figure 97). *UTC correction* is necessary to plot the data in local time.

Data gaps will be coloured with the background colour (6.4). You can interpolate data gaps by checking the box *Fill gaps by interpolation*.

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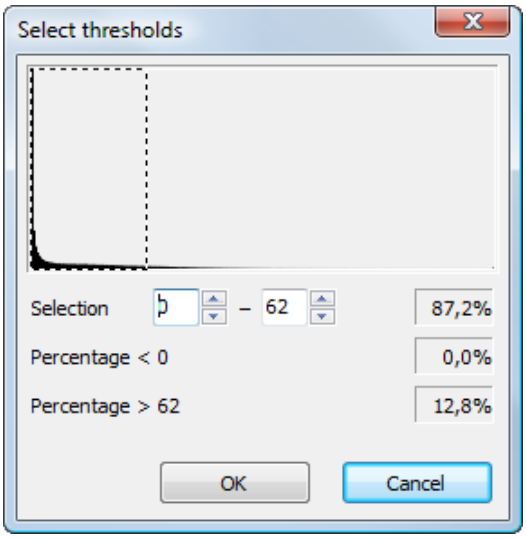

Figure 26: Histogram to select activity thresholds

Note: There are two common reasons for data gaps: (1) In older collars, data gaps occur when the hardware is busy (e.g. due to GPS positioning). (2) If you use activity mode 1, there will be periodical data gaps due to the protocol of data storage. Activity Pattern is able to automatically interpolate such data gaps. This can help you to interpret the activity pattern even if small data gaps are present, but in case of longer data gaps it will result in a false pattern. Please refer to Part II, Chapter 3-4 for details on the origin of data gaps and the algorithm of interpolation.

6.1 Canvas

Figure 27: Canvas section

To changes size of the actogram, use the *Canvas* section (Figure 27). Size is defined in pixels. Check the *Keep aspect ratio* box to keep the size relations of the actogram.

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The section *Labels* defines the captions of the axes (Figure 28).

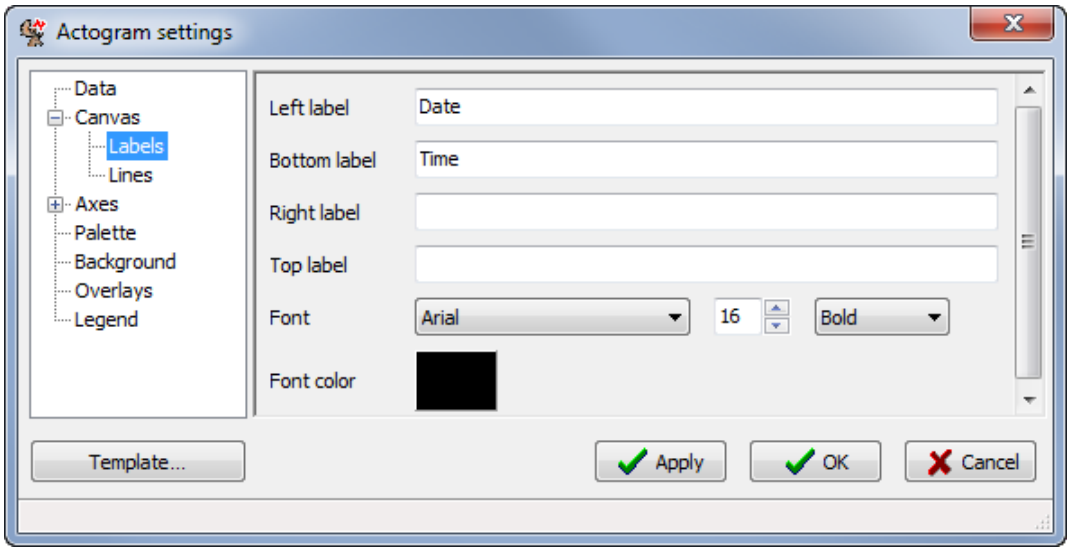

Figure 28: Labels section

By default the left label is “Date” and the bottom label is “Time”, but you can type in any text you wish. The labels are independent from the axes and will be visible even if the axes are not. Select font, size and format with *Font*. You can choose all fonts available on your computer. The default font colour is black, but it can be changed by double-clicking on the box next to *Font color*.

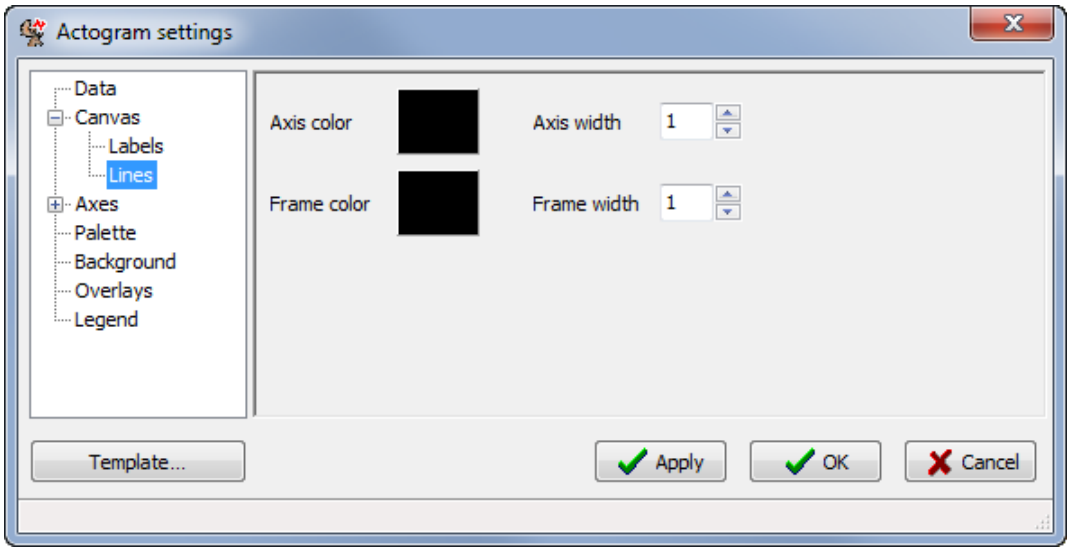

Figure 29: Lines section

Every side of the actogram is covered by default by an axis . If you disable an axis (see 6.2.1 and 6.2.2), a frame is displayed for this part of the actogram. You can change the colour of all

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axes and frames by double-clicking on the colour box in the *Lines* section to open a colour dialog (Figure 29), and thereby changing the number in the width box.

6.2 Axes

Adjustments to the axes of the actogram are made through the *Axes* section.

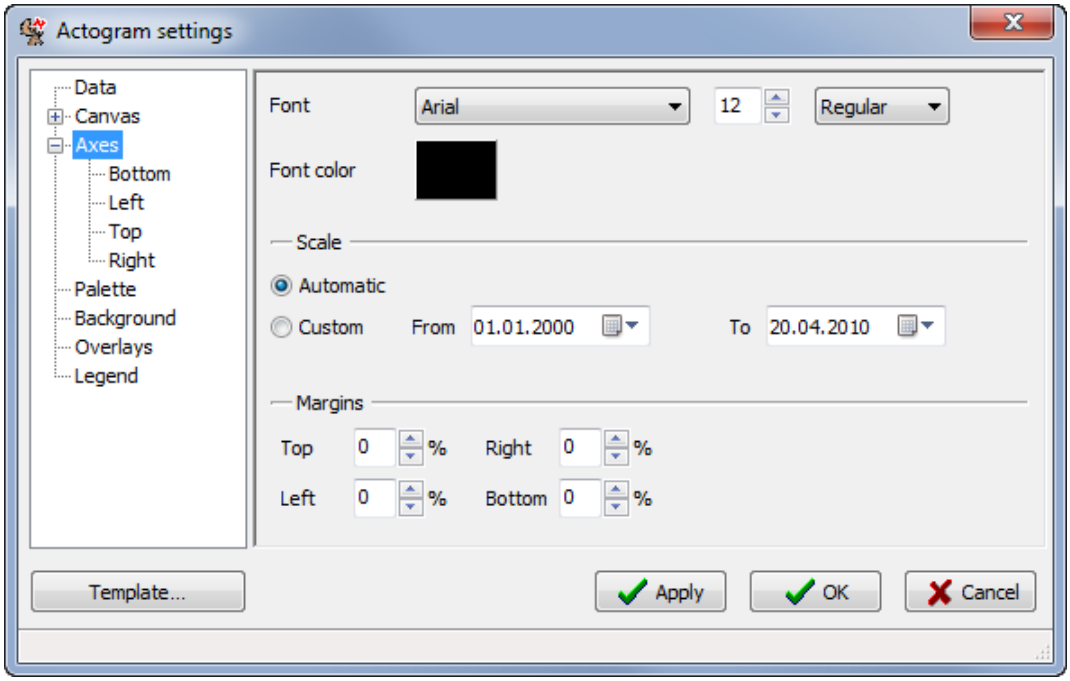

Figure 30: Axes section

You can change the font of the axes label, its size, style and colour. The colour dialog (Figure 20) can be accessed by double-clicking the box next to *Font color*.

By default, the axes show the exact time span of the data in the actogram selected in the *Data* section. If you want the axes to show another, for example longer, time span than the actogram itself, you can change it with *Date range* by selecting the button *Custom* and then choosing the start and end date for the axes. This selection is useful if you want to compare actograms of different animals covering different time spans.

With the item *Margins* you can insert a free space between the axes and the activity data.

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6.2.1 Bottom / Top

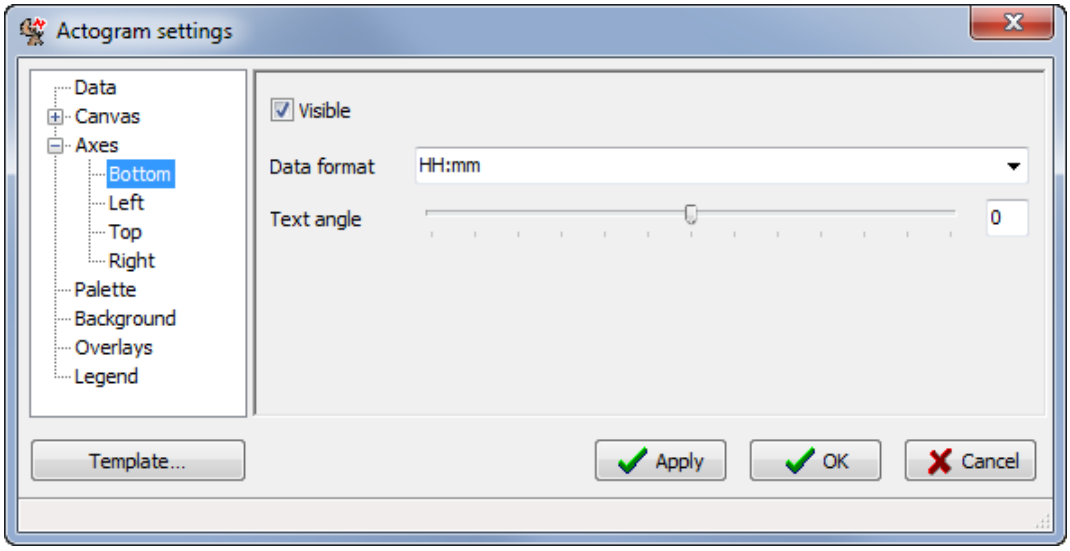

Figure 31: Section for bottom and top axis

The visibility of each axis can be set by selecting the axis and checking or unchecking the *Visible* box. Choose the *Data format* from the list of formats or type in one of the formats from Appendix II: Formats for date and time on actogram and chart axes. The *Text angle* can be changed either by moving the handle along the slider or by typing in a number.

6.2.2 Left / Right

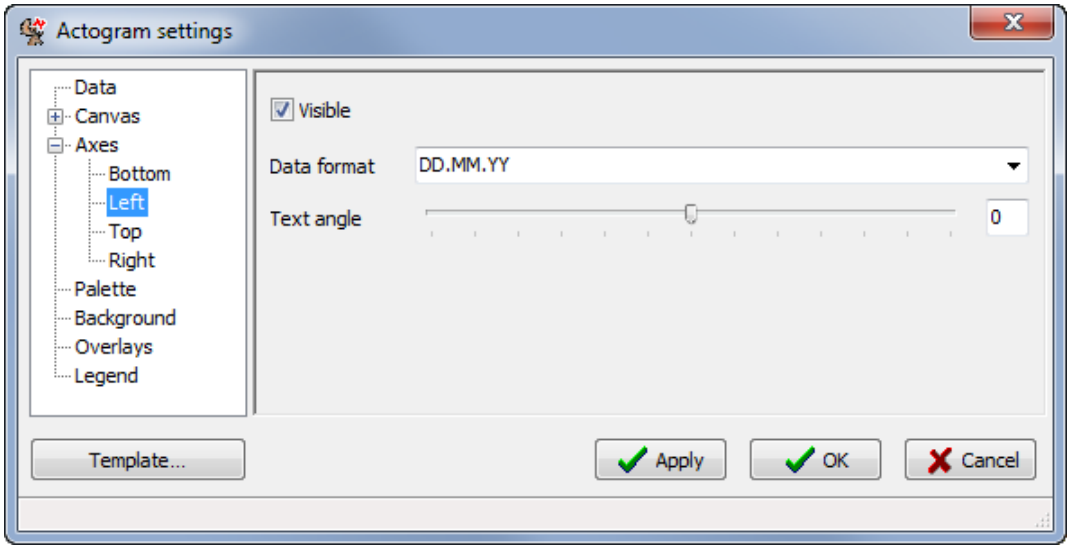

Figure 32: Section for left and right axis

The basic functions are similar to 6.2.1 *Bottom* / *Top*, but instead of a time you can choose dates for *Data format* (Appendix II: Formats for date and time on actogram and chart axes).

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6.3 Palette

You can change the colour scheme of the actogram with the *Palette* section.

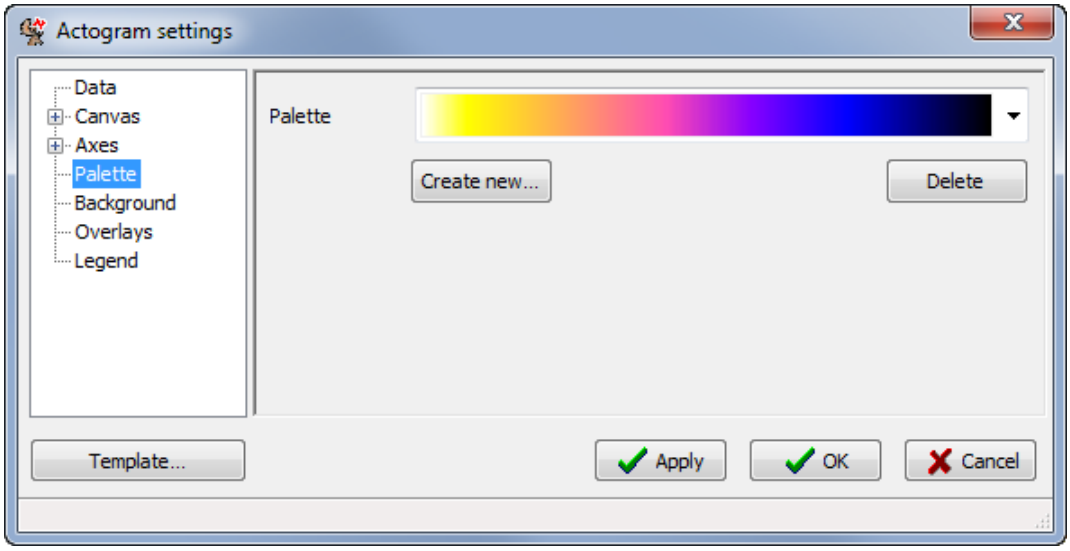

Figure 33: Palette section

There are three palettes provided by default. To delete a palette, select it and press Delete. To add a palette to the drop-down list, click Create new... to open a palette editing dialog (Figure 34).

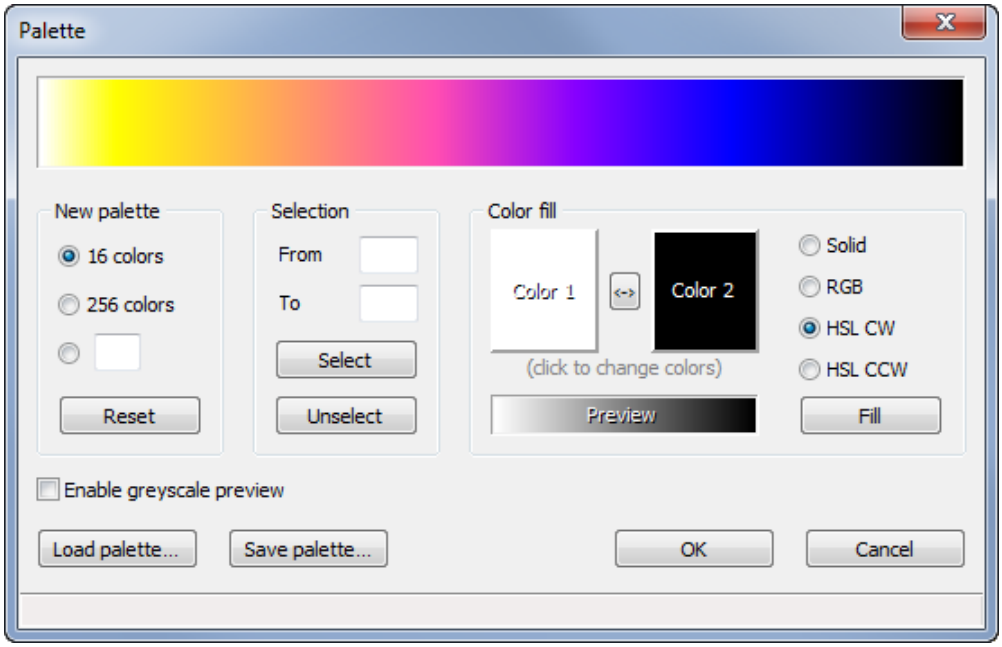

Figure 34: Palette creation dialog

The dialog's palette will be filled with the previously selected one, which you can modify now. In *New palette* you can choose between 16, 256 or a user-defined number of colours.

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Clicking *Reset* changes the number of colours while trying to keep the existing colour gradient. *Selection* allows you to select a range of colours by typing in the numbers of the first and last colours box in the palette preview (0-15 or 0-255) or by clicking and dragging with the cursor.

You can select the colours of new palette with *Color fill*. By double-clicking the boxes Color 1 and Color 2 you can open a colour selection dialog (Figure 20). The colour gradient defines how the entries between colours 1 and 2 are interpolated. Four options are available:

Solid	colourises the selection with colour 1	
RGB	straight gradient between colour 1 and 2	
HSL CW	clockwise rainbow gradient:	
HSL CCW	counterclockwise rainbow gradient:	

The gradient will first be shown in the *Preview*. Fill transfers the specified gradient into the selected part of the palette. If nothing is selected, the complete palette will be replaced. Check *Enable greyscale preview* to get a preview for the chosen palette in greyscale printing.

With Load palette... you can open an existing palette, Save palette... will save the current palette as Microsoft Palette file PAL in a place of your choice.

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6.4 Background

The *Background* section allows you to choose the colour for those parts of the actogram not filled with data (excluding the margins). Double-clicking the box next to *Fill color* let you access the colour dialog (4.1.2).

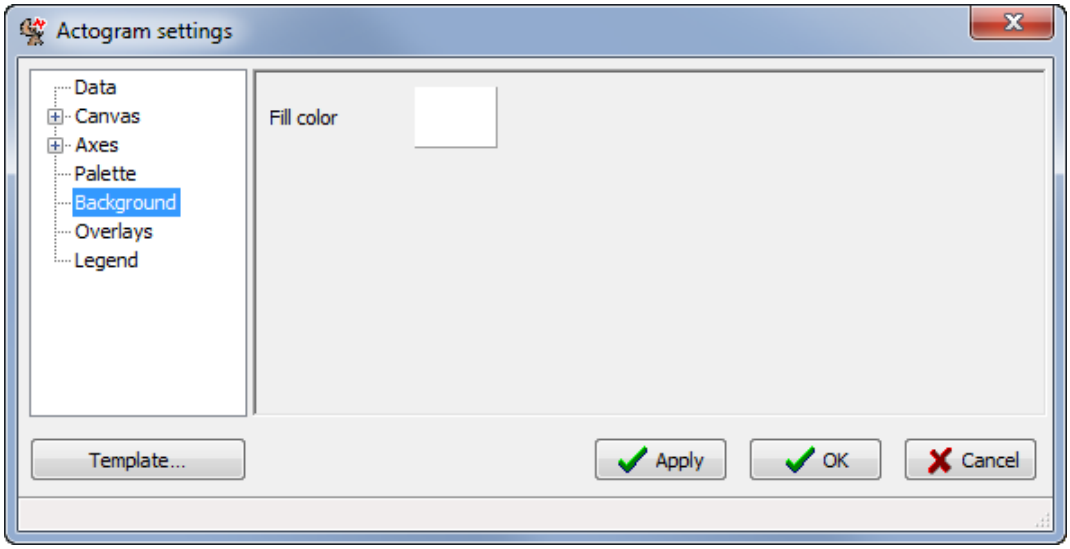

Figure 35: Background section

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6.5 Overlays

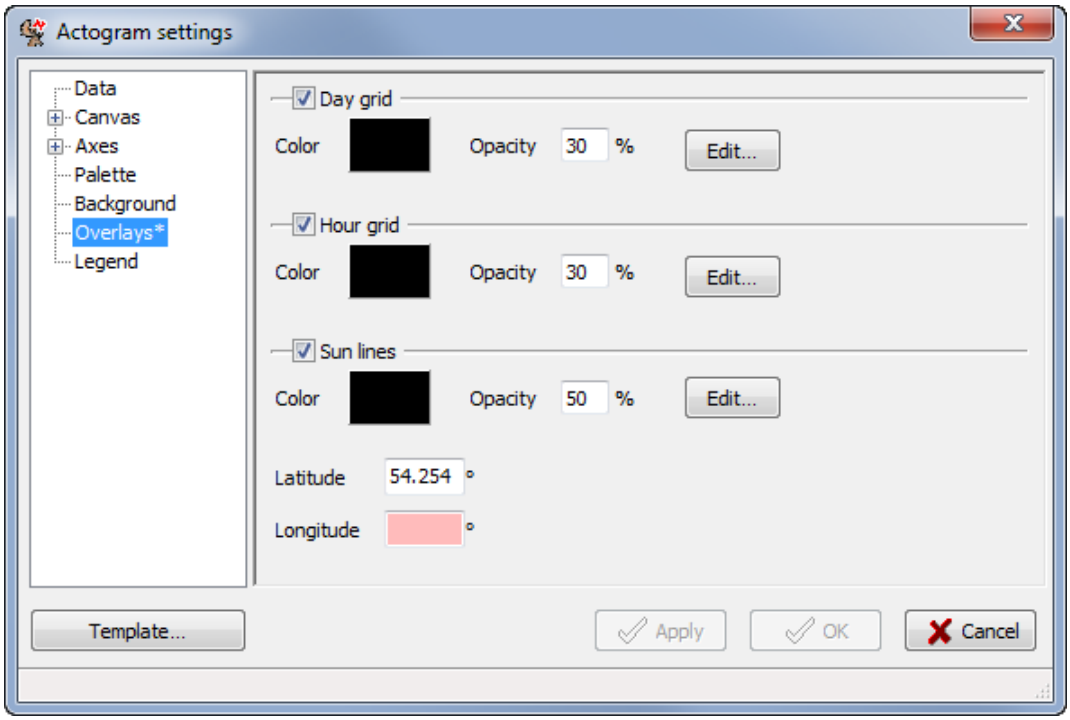

Figure 36: Overlays section

For better orientation, you can add time information as overlays to the actogram. You can insert horizontal day grids, vertical hour grids and sun lines by checking the boxes. By double-clicking the colour boxes, you can open the colour dialog (Figure 20). *Opacity* controls the intensity of the grid or sun lines.

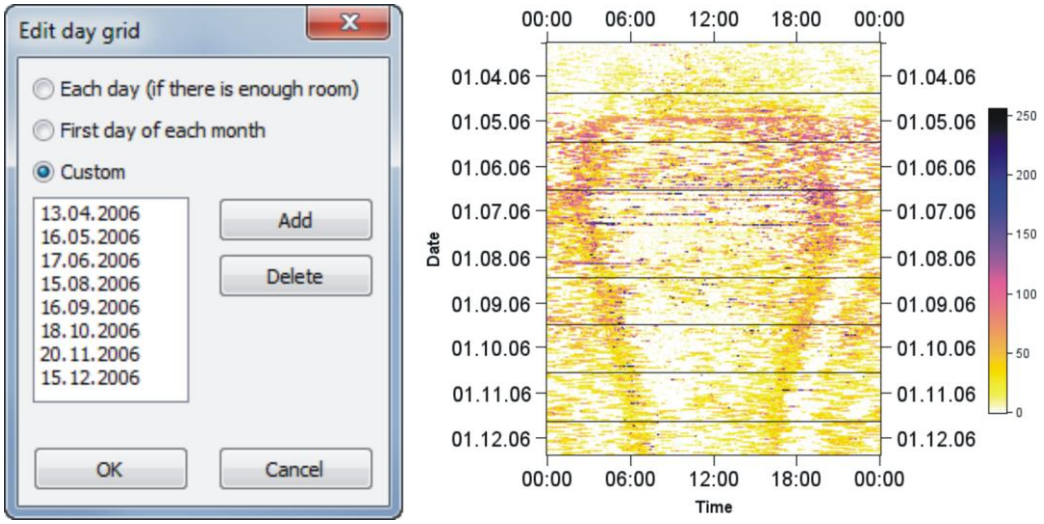

Figure 37: Day grid dialog (left); actogram with day grid (right)

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Horizontal day lines can be inserted for every single day if resolution allows so. Alternatively you can insert a line at the first day of each month. By checking Custom and pressing Add, you can select any date for the grid from the calendar function and add it to the list. After adding a new date, you can edit it by double-clicking it to start editing and clicking somewhere else to finish. Delete a date by selecting it with the mouse and press Delete.

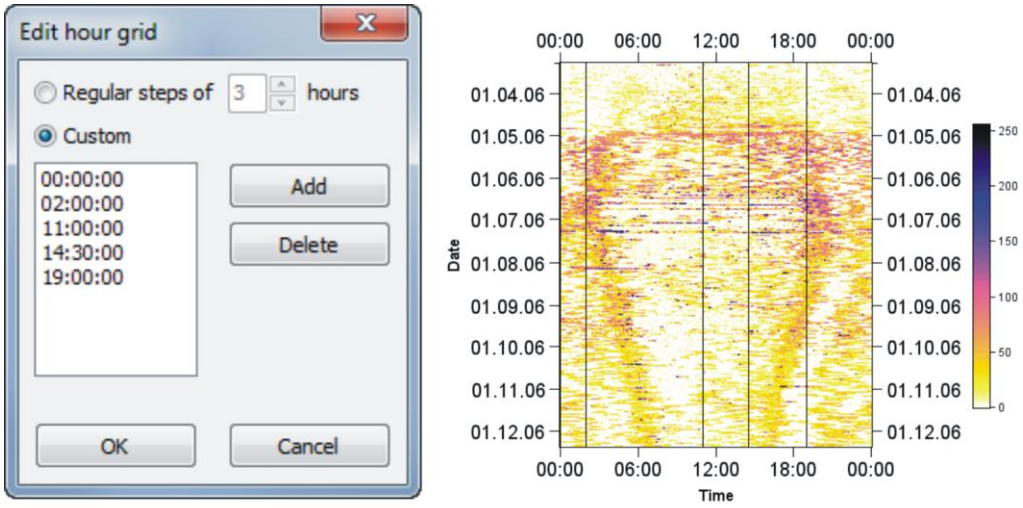

Figure 38: Hour grid dialog (left); actogram with hour grid (right)

Vertical hour lines can be inserted every full hour by checking *Regular steps of* N *hours*. For a user defined grid, check *Custom* and select times in the time box. Use Add to add them to the list and Delete to remove them.

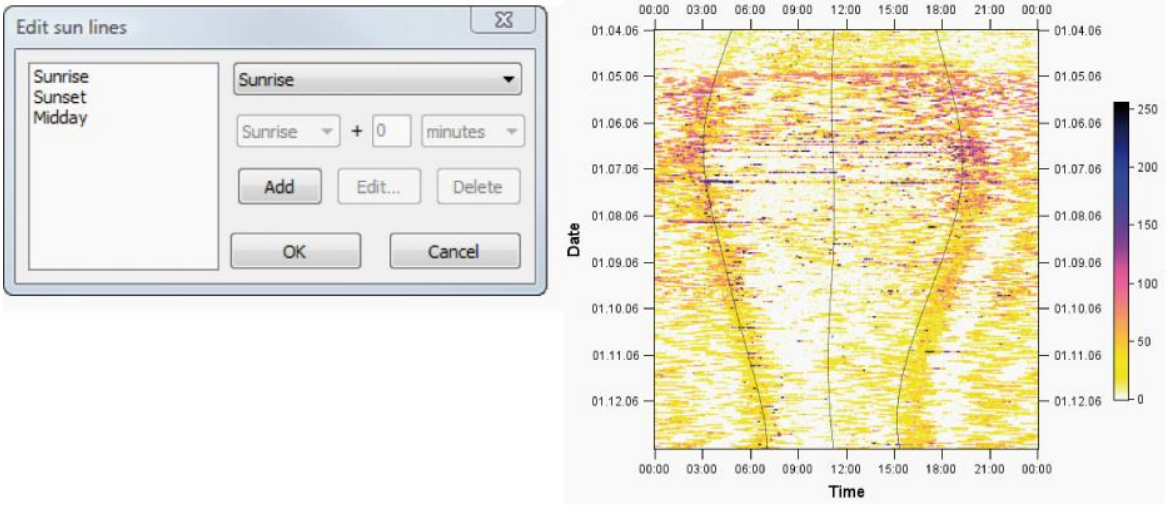

Figure 39: Actogram with sun lines for sunrise, midday and sunset

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To insert information about sunrise, sunset and twilight, you can add *Sun lines*. Latitude and longitude have to be typed in as decimal fraction (see Figure 36). Following sun positions are available:

Sunrise/sunset	defined as the instant at which the upper edge of the sun appears/disappears above the horizon.
Astronomical dawn/dusk	defined to begin in the morning and end in the evening when the sun is geometrically 18° below the horizon. At this stage sky illumination by the sun is too faint to be perceived.
Nautical dawn/dusk	defined to begin in the morning and end in the evening when the sun is geometrically 12° below the horizon. At this time, the outlines of ground objects might be distinguishable for humans. Most mammals should be able to see properly at this time.
Civil dawn/dusk	defined to begin in the morning and end in the evening when the sun is geometrically 6° below the horizon. At this time illumination is sufficient for humans to clearly distinguish their surroundings, but the horizon is still clearly defined and the brightest stars are visible under good weather conditions.
Midday	transit time of the sun through an imaginary line in the sky. Due to the advent of time zones, midday is approximately, but rarely exactly 12:00, but depends on longitude, latitude, altitude, and time of the year rather than on a time zone.
Midnight	time 12 hours after midday when the night is equidistant from dusk and dawn. Same as midday its actual time is not 00:00 but depends on longitude, latitude, altitude, and time of the year.

Sunrise and sunset are plotted by default. To add other lines, choose them from the top box. Select the parameter and press Add to add it to the left box. You can delete a change selected parameter with the Delete. To exchange a parameter, press Edit..., it will automatically change to Save. Choose the new parameter from the top list and press Save.

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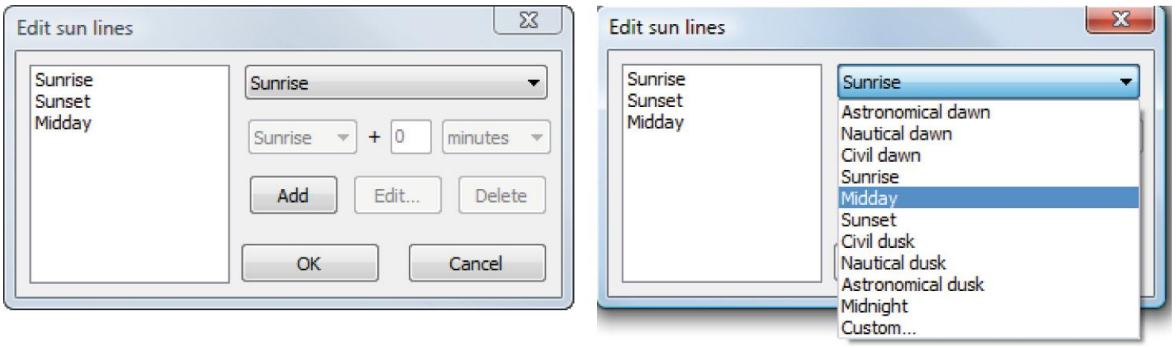

Figure 40: Sun lines dialog (left) and selection box for parameter (right)

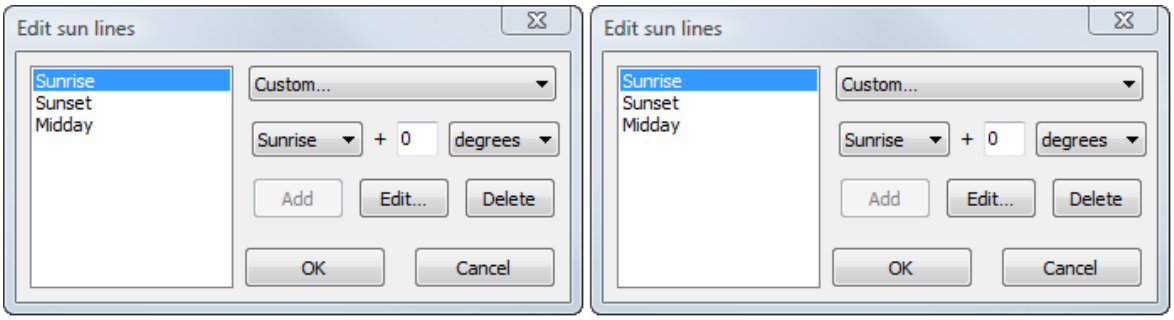

Figure 41: Sun lines dialog with Custom… selection

You can also insert lines with any relation to sun position you like by selecting *Custom…*. Then select sunrise, midday, sunset and midnight plus a user defined number of minutes or degrees. Degree offsets are only available for sunrise and sunset. Positive offsets mean a lower position of the sun (farther from the midday position).

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6.6 Legend

The *Legend* section controls the legend displayed to the right of the actogram.

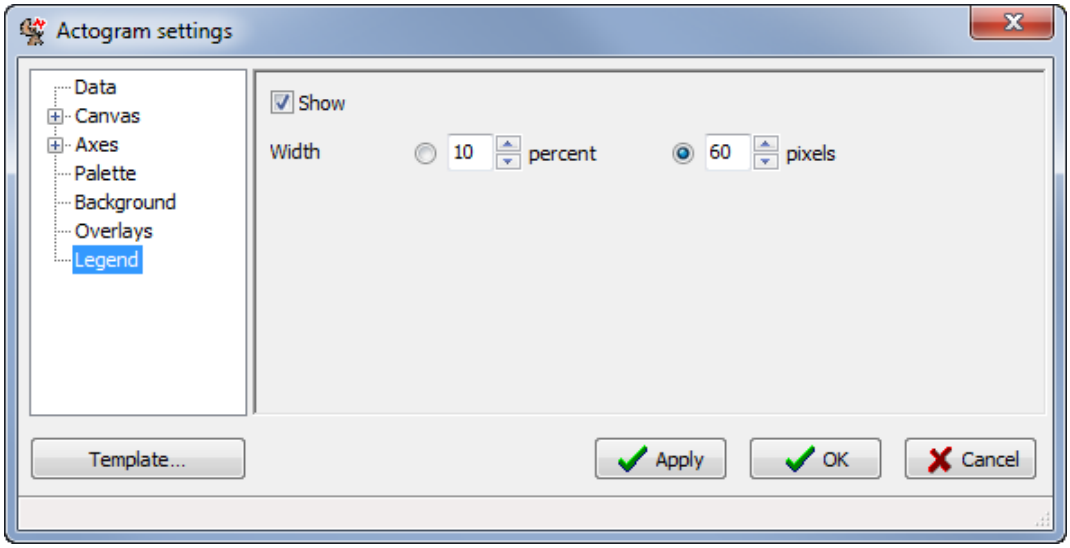

Figure 42: Legend section

Uncheck the *Show* box to hide the legend. Using the boxes next to *Width*, you can specify the width of the legend either in *percent* of the complete diagram, including actogram and legend, or in *pixels*. Both parameters will automatically change the width of the actogram. To change the width of the complete chart, use the *Canvas* section (Part I, Chapter 6.1).

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7 Statistical chart by date

Statistical by date (shortcut Ctrl + 2) allows you to calculate mean and median of activity for consecutive days or months (Figure 43). For the chart you can choose between lines or bars.

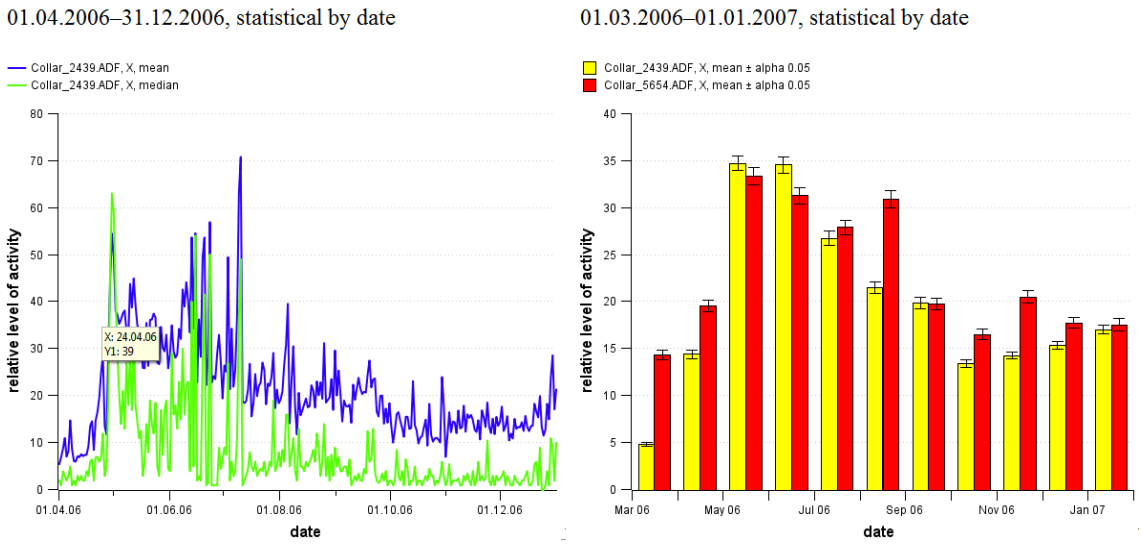

Figure 43: Statistical charts by date. Left: For one dataset, mean activity (blue line) and median of activity (green line) is given. Right: For two datasets, average monthly activity plus confidence interval α = 0.05 is given. The y-axis gives the relative level of activity as measured by the collar.

7.1 Data series

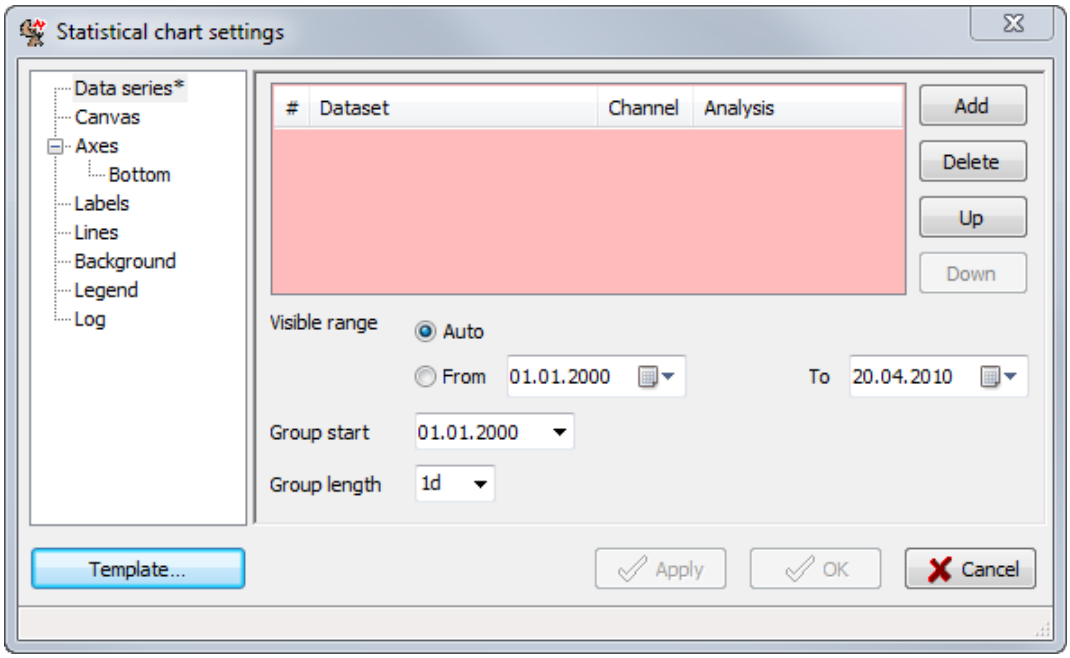

Figure 44: Data series section

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Select a previously loaded ADF file for the list with Add or delete an already chosen one with Delete (Figure 44). If you press Add, the window will automatically switch to the *Data series* section (Figure 45). Sections with missing compulsory input are indicated with an asterisk, fields with missing compulsory input are marked red.

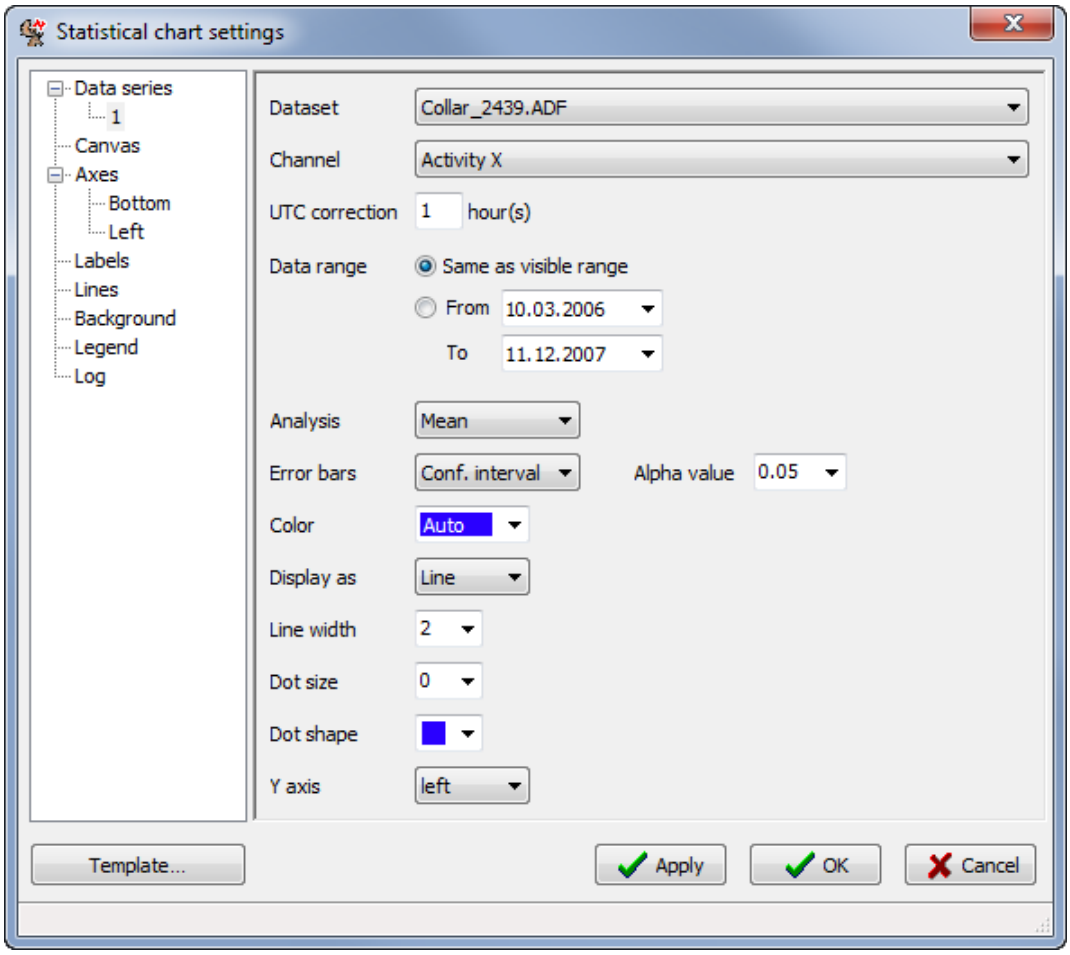

Figure 45: Window for choosing the settings for Statistical chart settings. The same section is shown for Statistical… by time and Rhythm analysis.

In the *Data series* subsection (Figure 45) select the *Dataset* and the *Channel* you want to analyse from the boxes. The selected file will be given a running number, which will appear as node for *Data series* in the left part of the window.

Add the *UTC correction in hours*. For the *Data range*, either check the button *Same as visible range* (default) or check the *From – To* boxes and use the calendar function.

For *Analysis*, you can choose between arithmetic Mean, Median or Count (number of values used for one data point) of data points per selected time group. For the Error bars, you can choose between confidence interval (Conf. interval), standard deviation (Std. deviation), standard error (Std. error) and no error bars (None). If you want confidence intervals to be

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shown, you can also choose between the *Alpha values* 0.05, 0.01 and 0.001; for the standard deviation you have the choice between different factors.

You can also choose the *Color* of the line with the colour box. Use the *Auto* selection, choose between 13 predefined colours or access the colour dialog with the *Custom…* button (Part I, Chapter 4.1.2). With *Display as*, you can choose to display the results as line or as bar chart.

You can also change the width of the line (*Line width*) and select dots to mark the values with *ot size* and *Dot shape*. The dots will always have the same colour as the line. You can also choose if the *Y axis* for this line will be on the left or right side of the graph.

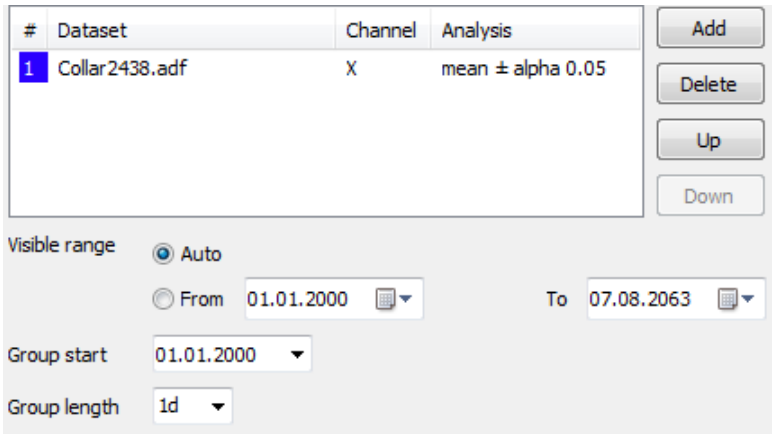

Figure 46: Data series section after one data series has been added

After these selections, return to the *Data series* section. You will now find the series you have just added in the list with a running number, the colour of the line, the analysed channel and a summary of the analysis. You can Add new ADF files to the list, Delete files or move them Up or Down in the list.

Choose the time period that will be plotted with *Visible range*. This setting is valid for all series in the list and will override the range selected for a single data series. With *Group length* you can choose the interval for your calculation, e.g. the mean activity per day, week, month or year. *Group start* allows you to choose the starting date for the groups. Data prior to this date but within the visible range will be plotted as well.

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7.2 Canvas

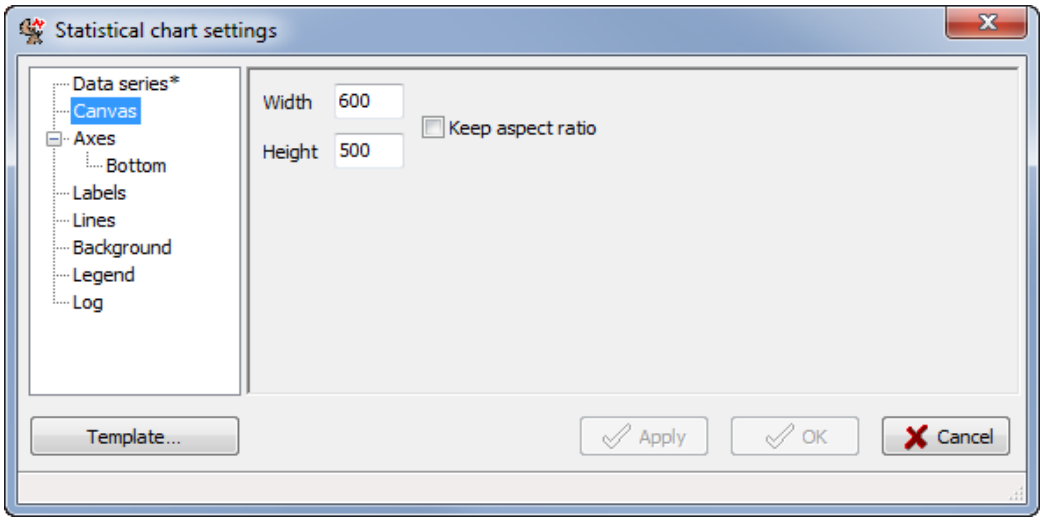

Figure 47: Canvas section

To changes the size of the chart, use the *Canvas* section. Size is defined in pixel. Check the *Keep aspect ratio* box to preserve the original proportion of the chart's height to its width.

7.3 Axes

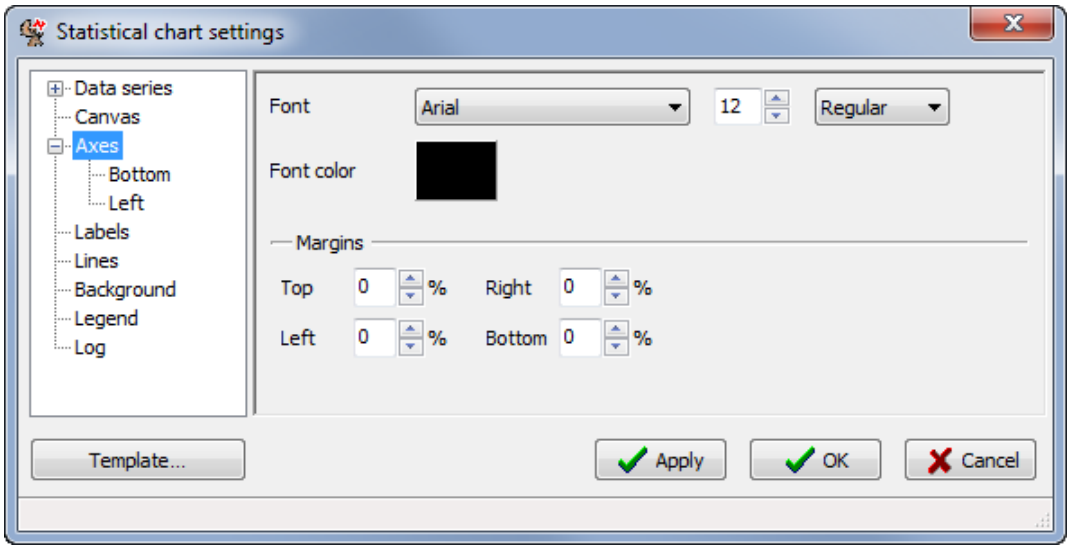

Figure 48: Axes section

The *Axes* section enables you to make changes on the charts axes. You can change the *Font*, its size, style and the *Font color*. You can also change the *Margins* of the chart. Margins are free space between the diagram and the surrounding graphical elements (axes, legend, title).

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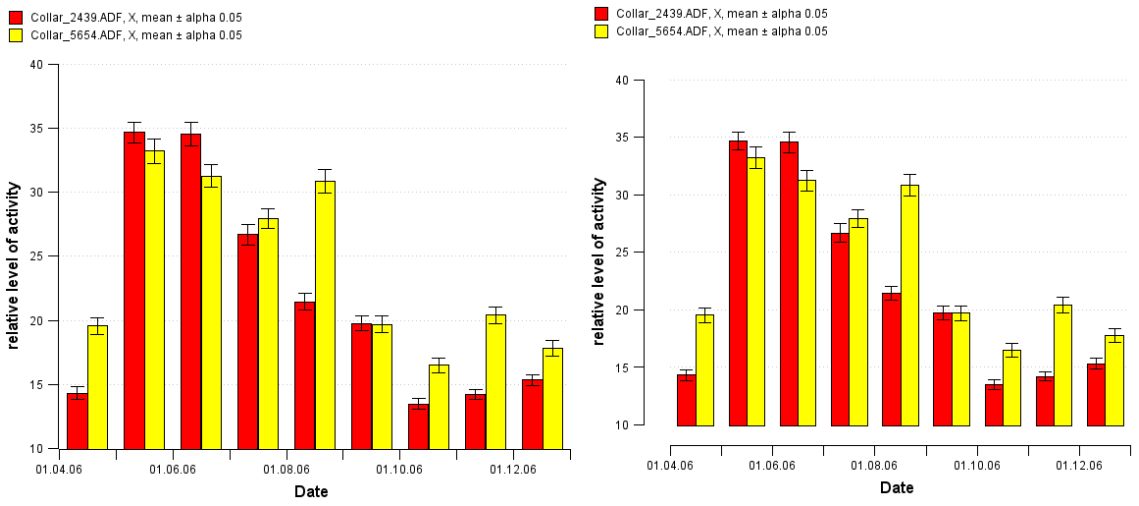

Figure 49: Chart of mean monthly activity of two individuals. Left: default layout. Right: with margins 5 % of chart size.

7.3.1 Bottom

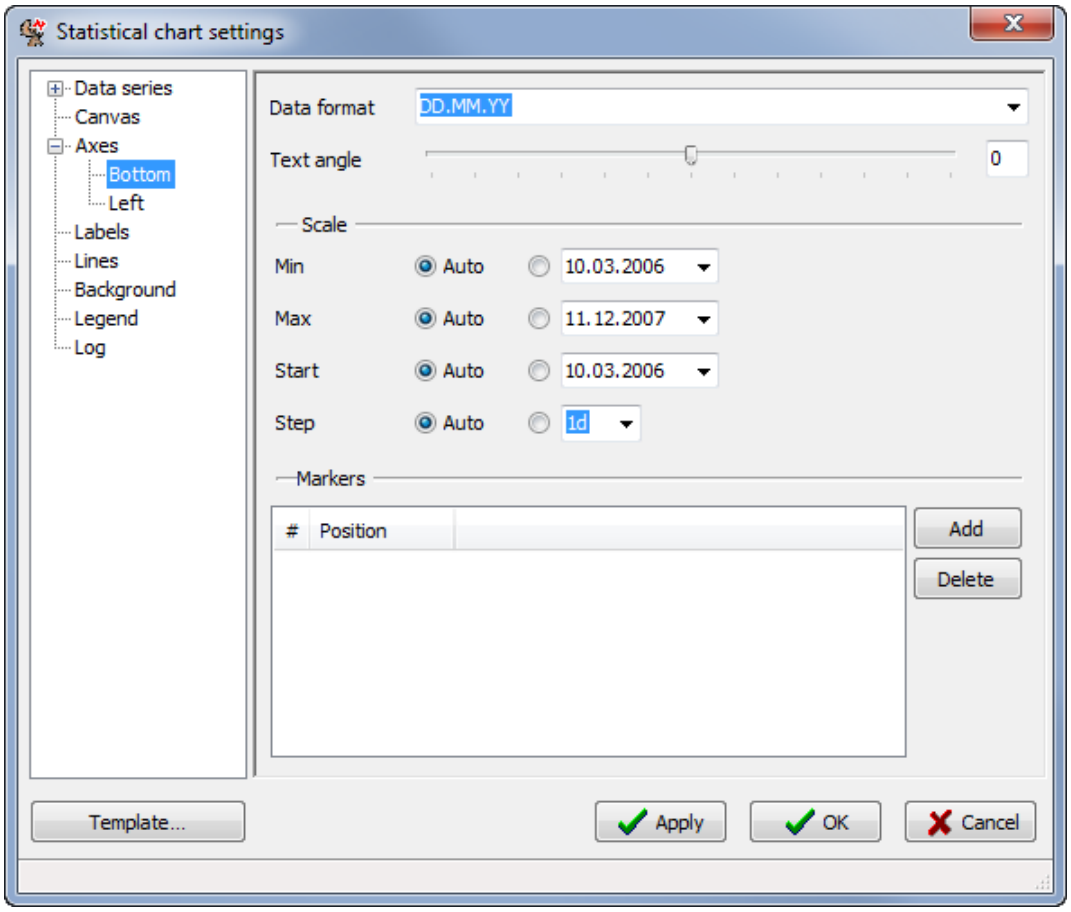

Figure 50: Bottom axis section

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Bottom allows you to change the appearance of the bottom axis. Change the *Data format* of the label by either selecting the preferred format from the list or by typing in a format of your choice.

To change the *Text angle* of the labels on the bottom axis either move the slider with the mouse or type the angle into the right box and press Enter. *Scale* changes the value range and subdivision steps of the axis. *Min* defines the first date on the axis, *Max* the last. The date from which the scaling is counted is given with *Start*. The scaling takes place in both directions. *Step* changes the intervals on the axis, in which the scaling is counted from the starting date.

With *Markers*, you can insert vertical lines to mark certain days. Press Add to enter the calendar function. By default, the 01.01.2000 will be given, but you can change it using the calendar function or typing in the required date. To change an entered position, double-click on it. Delete erases the position.

7.3.2 Left

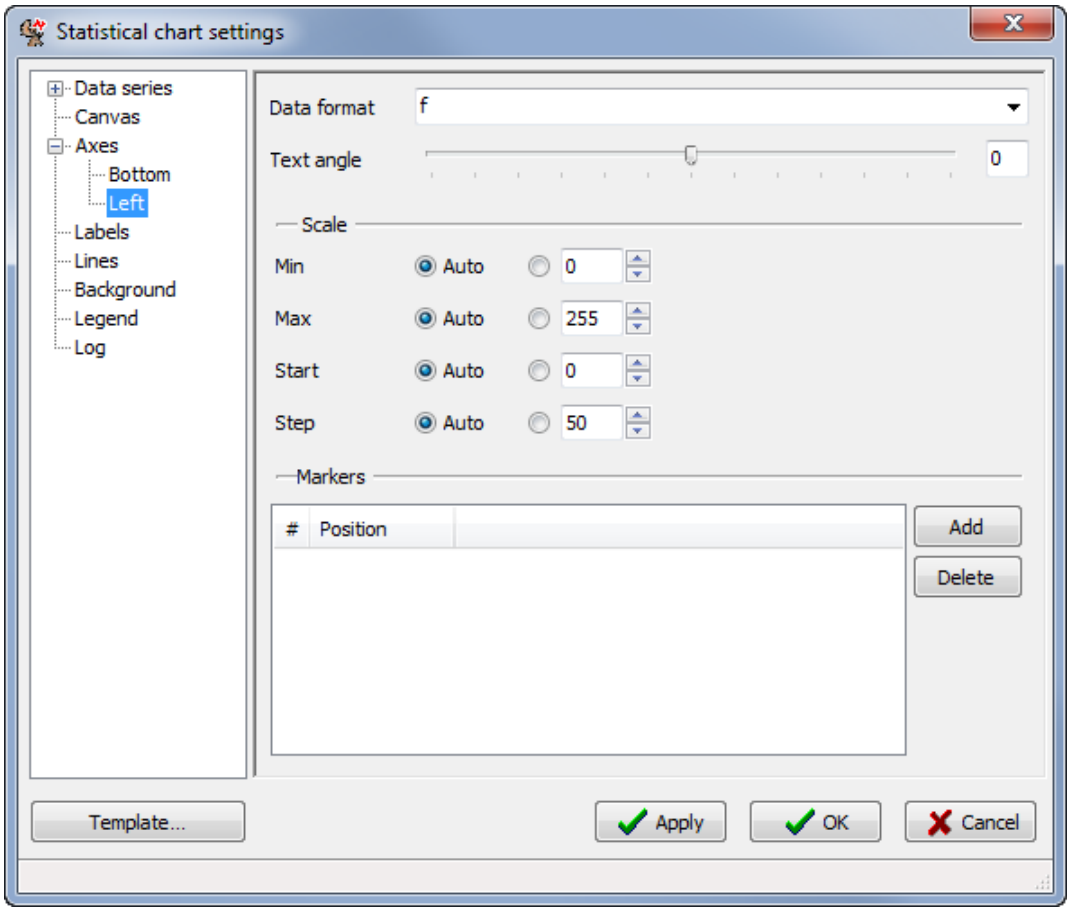

Figure 51: Left axis section

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To format the Y axis, select *Left*. With *Data format* you can select the number of decimal places. *f* indicates none, *fff* indicates two decimal places. You can also change the *Text angle* of the numbers by moving the slider or by typing the angle into the right box and pressing Enter. The *Scale* can be selected automatically with *Auto*. Alternatively, you can select a minimum (*Min*) and maximum (*Max*) value. If any calculated data point of the series lies outside of the range specified by *Min* and *Max*, the range will be expanded sufficiently. *Start* defines a value from which the *Steps* for the scaling are counted.

Horizontal lines can be inserted with *Markers*. Press Add to add a new position and type in the desired value. To change a value, double-click on it. Delete erases the position.

7.4 Labels

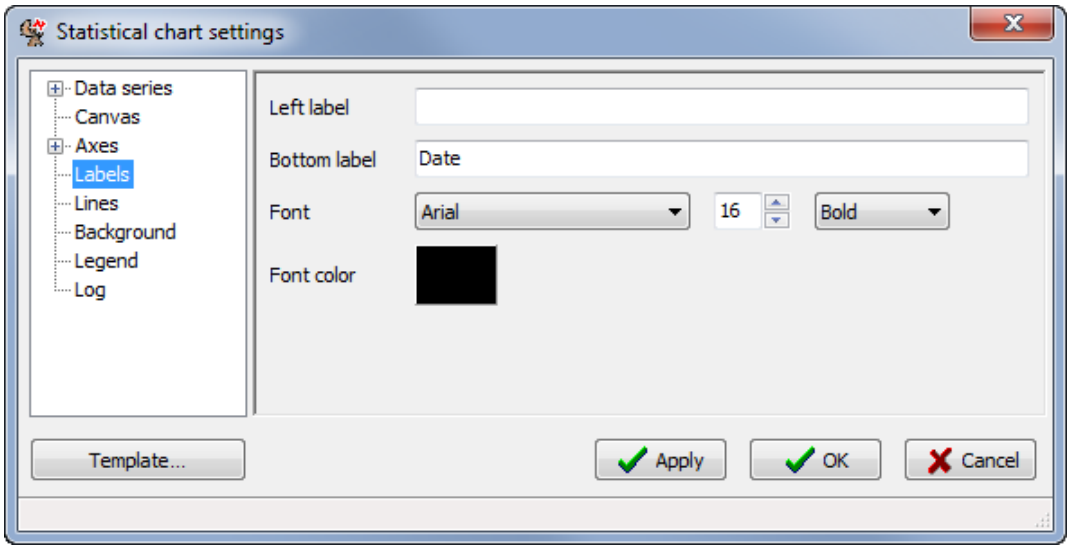

Figure 52: Labels section

To change text and format of an axis labels, use *Labels*. To insert a label type the text you would like to appear next to the axis label into the *Left label* or *Right label* box, respectively. A text box for the axis label will only be displayed if there are data series associated with that axis, which is done under *Data series* ⇨ *1* ⇨ *Y axis*.

By default, the X axis is labelled as *Date* (Statistical chart by date) or as *Time* (Statistical chart by time). Use the *Bottom label* box to change this label. You can also change the *Font*, its size, style and the *Font color* (double-click on coloured box).

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7.5 Lines

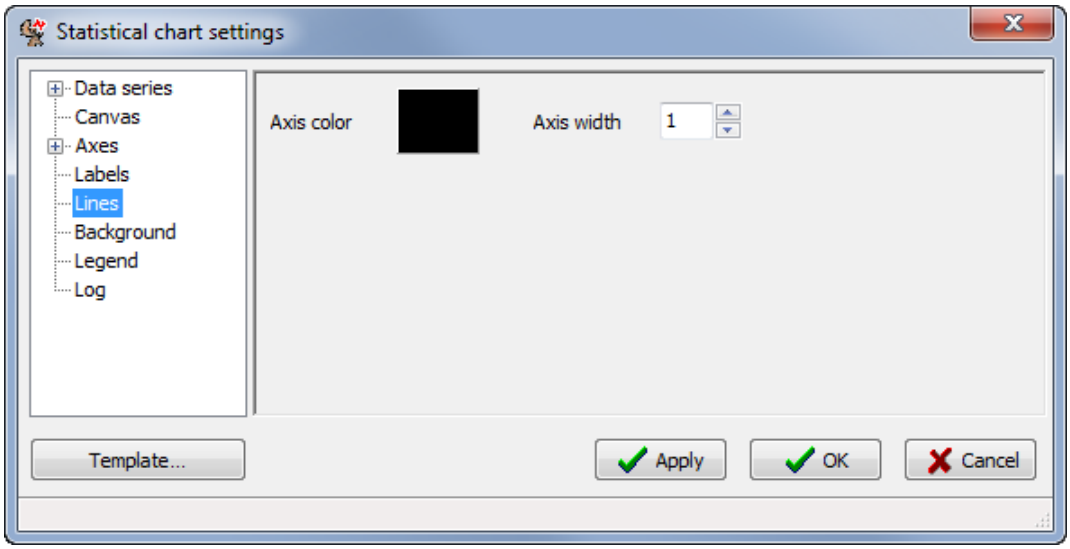

Figure 53: Lines section

In the Lines section, you can change the *Axis* colour using the *color selection* dialog and the *Axis width* given in pixels.

7.6 Background

The *Background* section is identical to the Actogram-Background section, so please refer to 6.4.

7.7 Legend

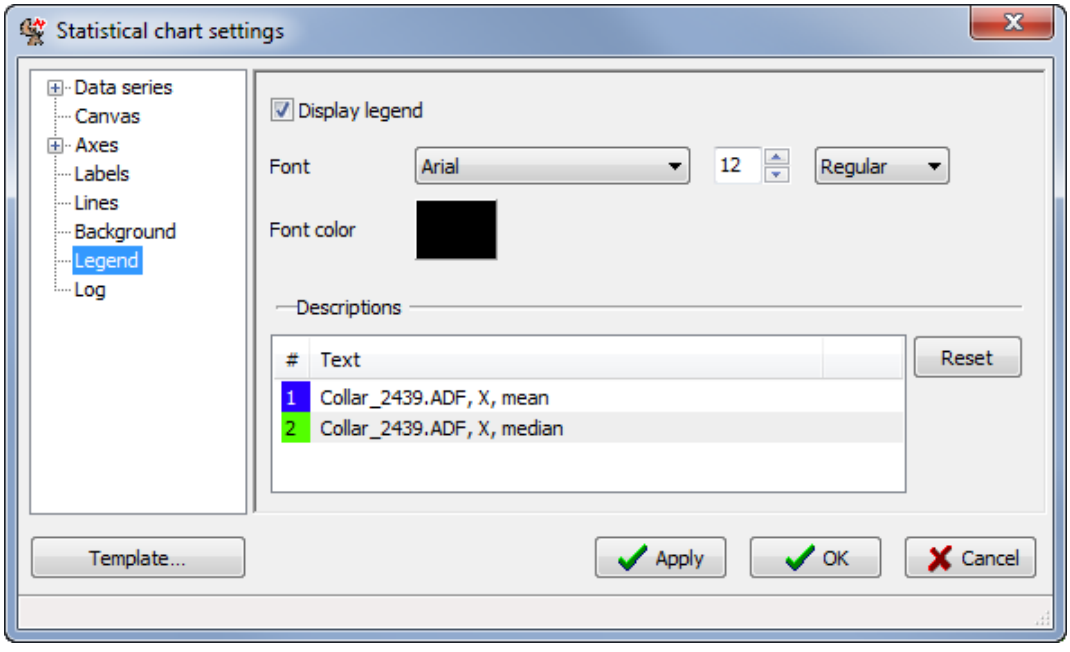

Figure 54: Legend section

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By default, a legend will be displayed on top of the chart with the filename, the channel and the analysis. You can hide this legend by unchecking *Display legend* in the *Legend* section. In this section, you can also change the *Font*, its size, style and the *Font color* (double-click on coloured box).

You also have the possibility to change the text of the legend in the *Descriptions* list. By double-clicking on the text you can delete or change it. If the description exceeds a maximum length, only the first part of the text is displayed. It is not possible to insert a line break. To return to the default text, select the text, then press Reset and Apply or OK.

7.8 Log

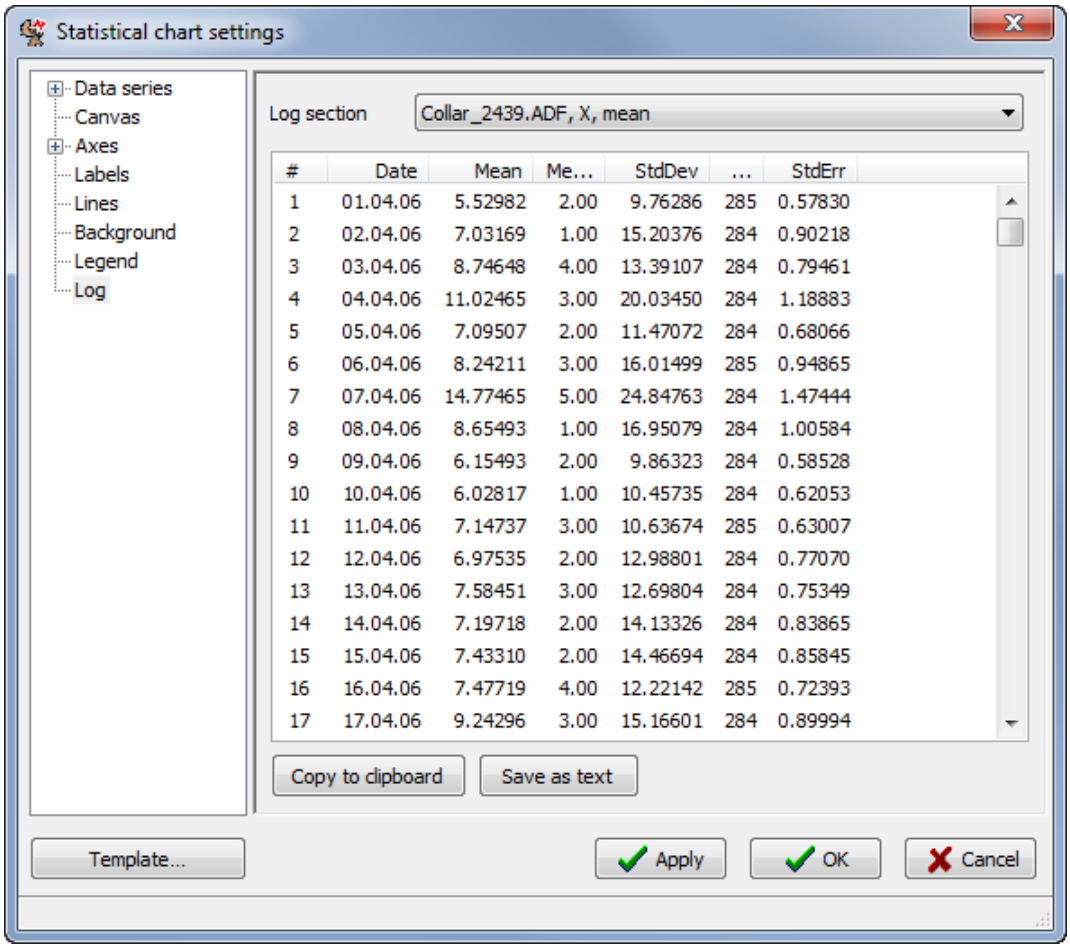

Figure 55: Log section

If you need the results shown in the graph for further analysis, you can access them in the log section. Select one data series from the list in the *Log section* box. Then, all data points will be shown in the window underneath with running number *#*, *Date*, *Mean*, *Median*, standard deviation (*StdDev*), *Count*, standard error (*StdErr*), *Lower bound* and *Upper bound* The latter give you the range of the lowest and highest value of the confidence interval or the standard deviation as selected by you in *Data series*.

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Activity Pattern performs each analysis separated for single datasets. Mean values for a group of individuals cannot be calculated. Copy to clipboard allows you to insert the data as a table into another program (e.g. MS Word, MS Excel, OpenOffice or a text editor) for further analysis. Save as text saves the data as a tab-delimited text file.

8 Statistical by time

Statistical by time (shortcut Ctrl + 3) allows you to calculate mean and median of activity for the course of the day.

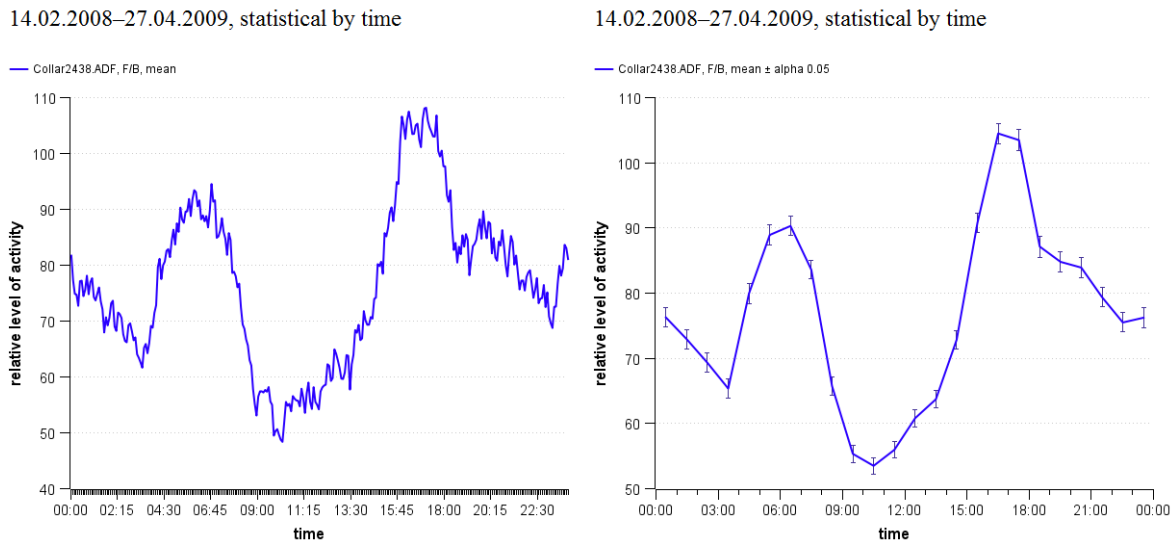

Figure 56: Statistical chart by time. Mean activity calculated in the course of the day for one month with an interval length (left) 5 minutes and (right) 60 minutes with confidence interval α = 0.05.

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8.1 Data series

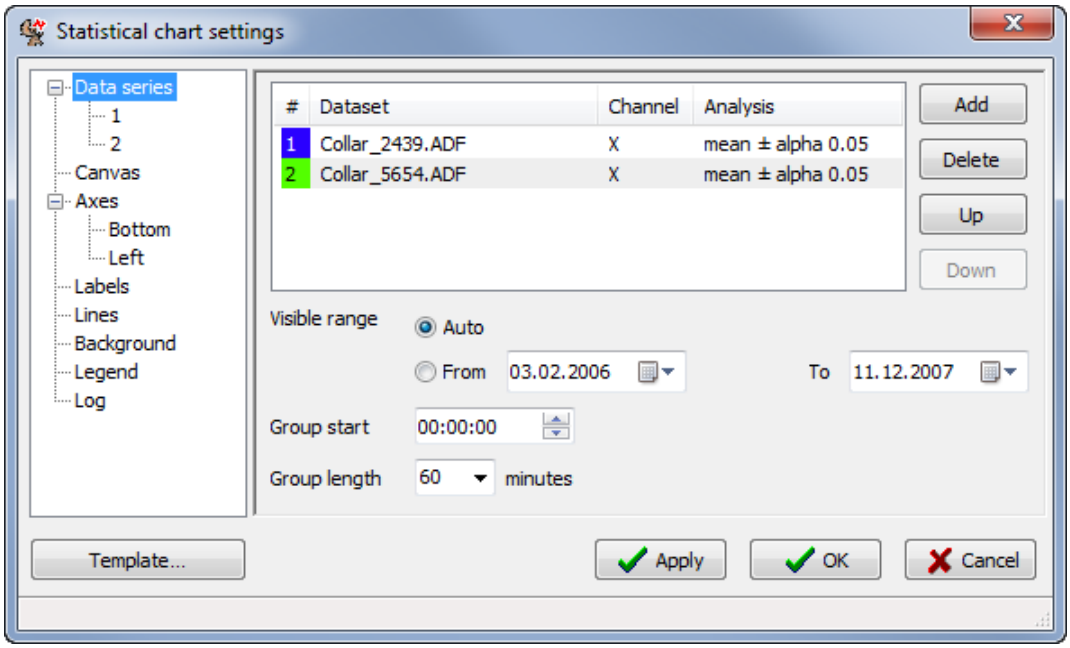

Figure 57: Settings window with list of ADF files for statistical chart by time

As in statistical chart by date, select a previously loaded ADF file for the list with *Add* or delete an already chosen one with *Delete*. If you press Add, the window will switch automatically to the *Data series* section (Figure 58). Proceed as described in Part I, Chapter 7.1.

After you have selected the analysis parameters, return to the *Data series* section (Figure 57). You will now find the selected file in the list with a running number, the colour of the line, the analysed channel and the analysis parameter. You can Add new ADF files to the list, Delete files or move them Up or Down in the list.

Choose the time period that will be plotted with *Visible range*. This setting is valid for all files in the list and will override the range selected for a single data series. With *Group start* you select the time of day your chart will start; by default it is midnight. With G*roup length* you select the interval for the analysis in this chart. The minimum interval is 5 minutes, the maximum 120 minutes. Longer intervals can result in a clearer pattern; however, smaller peaks are leveled out, and the overall structure of the activity pattern might be lost. If you choose an interval smaller than your sampling interval, the chart will show only the existing data points without a connecting line.

8.2 Canvas

See 7.2.

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8.3 Axes

See 7.3.

8.4 Labels

See 7.4.

8.5 Lines

See 7.5.

8.6 Background

See 7.6.

8.7 Legend

See 7.6.

8.8 Log

See 7.6.

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9 Rhythm analysis

Rhythm analysis (shortcut Ctrl + 4) performs an autocorrelation and a Fourier transform. Based on these, it calculates and plots the Harmonic Part and the Degree of Functional Coupling. For explanation of these analyses and the equations used, please refer to Part II, Chapter 4-7.

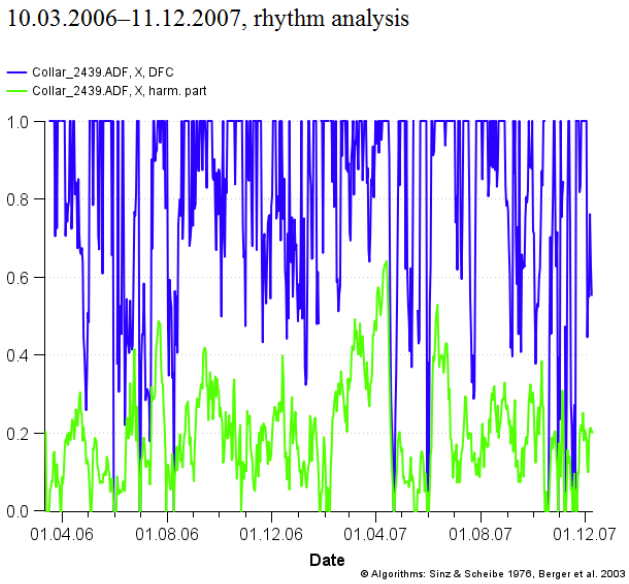

Figure 58: Chart with Harmonic Part (green) and Degree of Functional Coupling (blue), algorithms from Berger et al. (2003) and Sinz & Scheibe (1976).

9.1 Data series

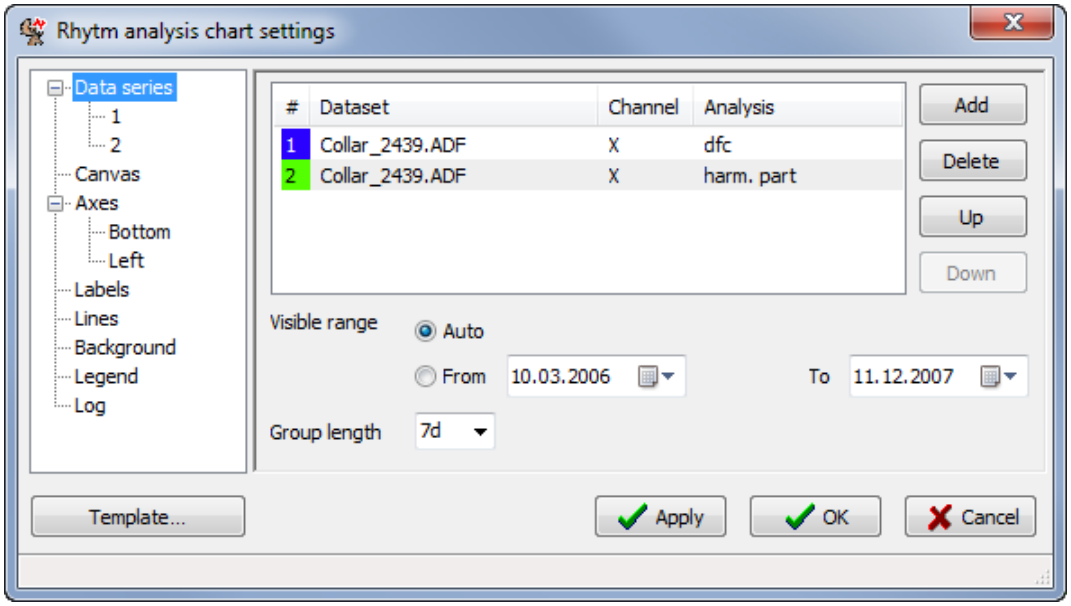

Figure 59: Data series section for rhythm analysis

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As described in 7.1, select a previously loaded ADF file for the list with Add or delete an already chosen one with Delete. If you press Add, the window will switch automatically to the series parameters section shown in Figure 60.

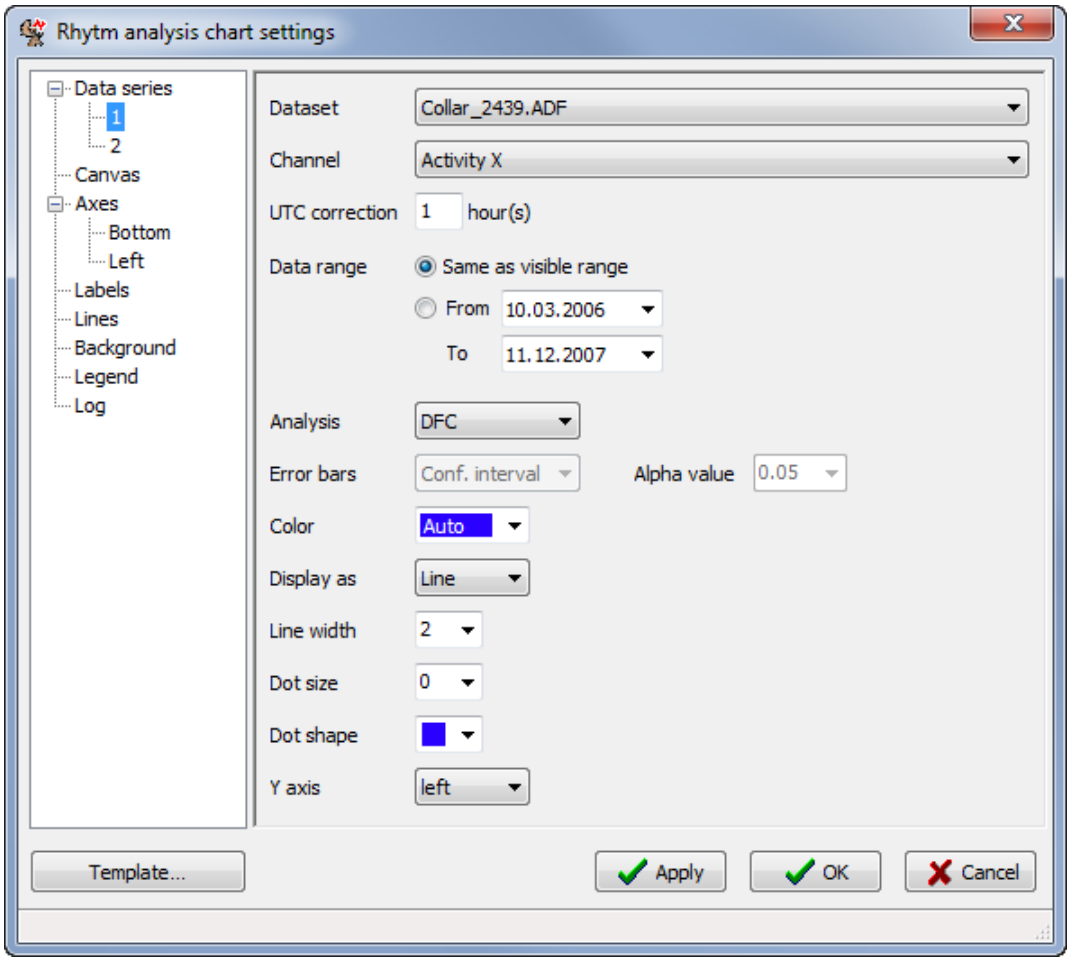

Figure 60: Window for choosing the settings for rhythm analysis

In the new section, select the *Channel* you want to analyse. In the next line, add the *UTC correction in hours*. For the *Data range*, either check the button *Same as visible range* (default) or check the *From – To* boxes and use the calendar function. You can also select the *Color* for the series with the colour box and the width of the line (*Line width*).

With *Analysis*, you can choose between Degree of Functional Coupling (DFC) and Harmonic Part (Harm. part). For comparison, you can also calculate the arithmetic Mean, the Median or the Count (number of values used for one data point) of data points per selected time group. For the *Error bars*, which are only available if one of the statistical analysis modes is selected, you can choose between confidence interval (Conf. interval), standard deviation (*Std. deviation*), standard error (*Std. error*) and no error bars (*None*). If you choose *confidence*

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interval, you can also choose between the *Alpha values* 0.05, 0.01 and 0.001; for the standard deviation you can choose between different factors.

Although the dialog is similar to the Statistical charts analysis dialog, analysis is slightly different. Harmonic Part and DFC will be calculated over 7 respectively 10 days, and so will be mean, median and count. The dates shown in the chart refer to the first day of the analysed group.

In this section, you can also choose the *Color* of the line with the colour box. Use the *Auto* selection, choose between 13 predefined colours or access the colour dialog with the *Custom…* button (4.1.2). With *Display as* you can decide if the results will be displayed as Lines or Bars. If you choose lines, you can change the width of the line (*Line width*) and select dots to mark the values with *Dot size* and *Dot shape*. The dots will always have the same colour as the line. You can also choose if the *Y axis* for this curve will be on the left or right side of the graph.

After these selections return to the *Data series* section (Figure 59). You will now find the selected file in the list with a running number, the colour of the line, the analysed channel and the analysis parameter. You can Add new ADF files to the list, Delete files or move them Up or Down in the list.

Rhythm analysis is calculated over several days. Therefore, in *Data series*, select the number of days to be analysed for one data points with *Group length*. From the preset list you can choose between 7 and 10 days, but you can type in every number of complete days you wish. An autocorrelation is then calculated over the given time period to exclude background noise. The suitable group length depends on the research question and the results will differ with the number of tested days. Please refer to Part II, Chapter 7.2.3 for background information on selecting the adequate group length. Select the range over which the groups will be calculated with *Visible range*. This setting is valid for all files in the list and will override the range selected for a single data series.

If you want to change the analysis or graphical parameters for a data series, select the ADF file from the list of loaded files in the *Dataset* tab by choosing its number in the left part of the tab or double-click on the list. This will switch to the *Data series* section (Figure 60).

9.2 Canvas

See 7.2.

9.3 Axes

See 7.3.

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9.4 Labels

See 7.4.

9.5 Lines

See 7.5.

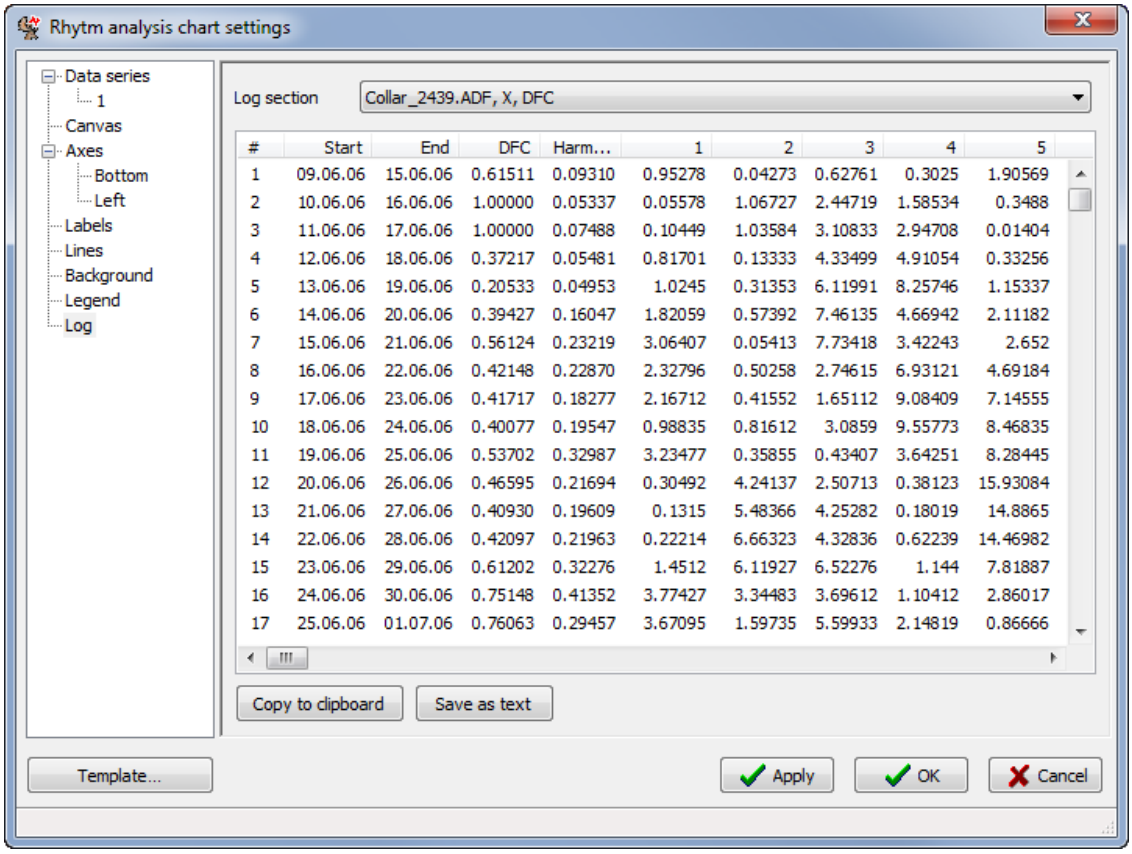
9.6 Background

See 7.6.

9.7 Legend

See 7.6.

9.8 Log



#	Start	End	DFC	Harm...	1	2	3	4	5
1	09.06.06	15.06.06	0.61511	0.09310	0.95278	0.04273	0.62761	0.3025	1.90569
2	10.06.06	16.06.06	1.00000	0.05337	0.05578	1.06727	2.44719	1.58534	0.3488
3	11.06.06	17.06.06	1.00000	0.07488	0.10449	1.03584	3.10833	2.94708	0.01404
4	12.06.06	18.06.06	0.37217	0.05481	0.81701	0.13333	4.33499	4.91054	0.33256
5	13.06.06	19.06.06	0.20533	0.04953	1.0245	0.31353	6.11991	8.25746	1.15337
6	14.06.06	20.06.06	0.39427	0.16047	1.82059	0.57392	7.46135	4.66942	2.11182
7	15.06.06	21.06.06	0.56124	0.23219	3.06407	0.05413	7.73418	3.42243	2.652
8	16.06.06	22.06.06	0.42148	0.22870	2.32796	0.50258	2.74615	6.93121	4.69184
9	17.06.06	23.06.06	0.41717	0.18277	2.16712	0.41552	1.65112	9.08409	7.14555
10	18.06.06	24.06.06	0.40077	0.19547	0.98835	0.81612	3.0859	9.55773	8.46835
11	19.06.06	25.06.06	0.53702	0.32987	3.23477	0.35855	0.43407	3.64251	8.28445
12	20.06.06	26.06.06	0.46595	0.21694	0.30492	4.24137	2.50713	0.38123	15.93084
13	21.06.06	27.06.06	0.40930	0.19609	0.1315	5.48366	4.25282	0.18019	14.8865
14	22.06.06	28.06.06	0.42097	0.21963	0.22214	6.66323	4.32836	0.62239	14.46982
15	23.06.06	29.06.06	0.61202	0.32276	1.4512	6.11927	6.52276	1.144	7.81887
16	24.06.06	30.06.06	0.75148	0.41352	3.77427	3.34483	3.69612	1.10412	2.86017
17	25.06.06	01.07.06	0.76063	0.29457	3.67095	1.59735	5.59933	2.14819	0.86666

Figure 61: Log section

The numerical results of the rhythm analysis can be accessed in the log section of the settings dialog. Select one data series from the list in the *Log section* box. Then, all data points will be shown in the window underneath with running number *#*, *Start* and *End* of the calculated period, *DFC* (only shown if included in analysis) and Harmonic Part (*Harm. part*). The complete periodogram for each data group is given in columns 1, 2 etc. The column headers

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contain the divisor by which the window length is to be divided to get the period length for this column. For example, when the window length is set to 7 days, column number 7 will contain the 7 / 7 = 1 day = 24 hours period, column 14 the 12 hours period etc.

Copy to clipboard allows you to insert the data as a table into another program (e.g., MS Word, MS Excel, OpenOffice or a text editor). Save as text saves the data as a tab-delimited text file.

10 Cosinor

Cosinor (shortcut [Ctrl + 5]) performs a mathematical procedure to test a time series for conformance with a cosine wave. For details on this analysis, please refer to Part II, Chapter 4-6.

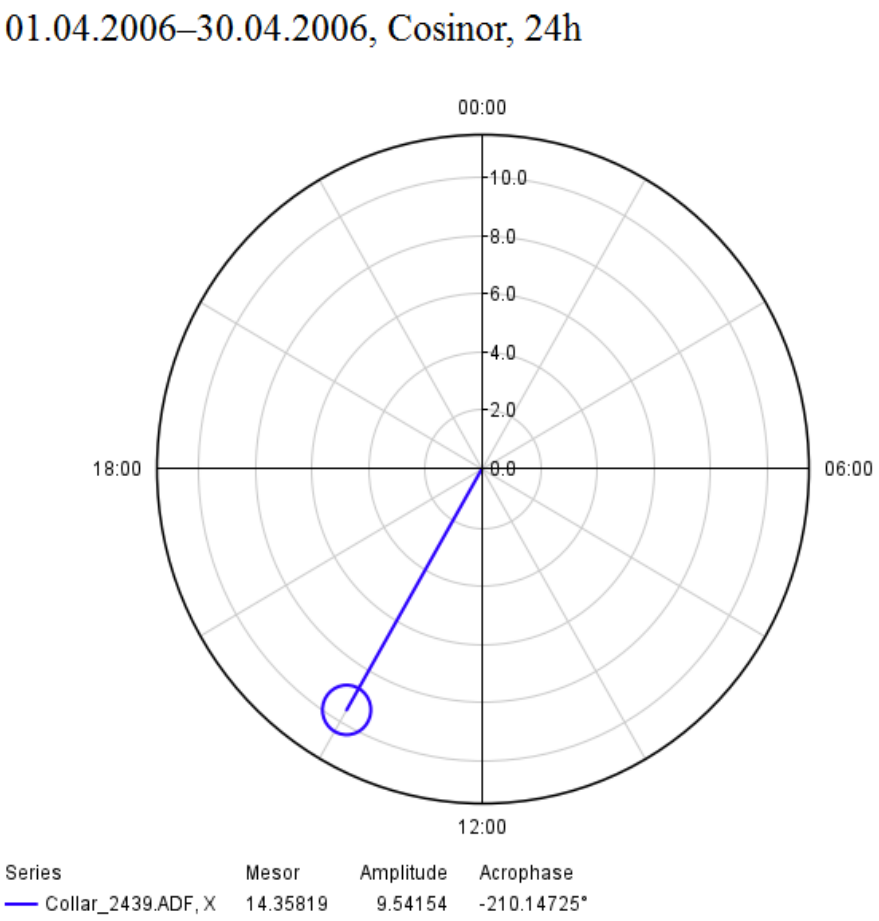

Figure 62: Cosinor-Plot for the 24 hour period. The blue line represents the vector of the cosinor with the direction showing the phase angle between the onset of the period and the acrophase (in this case equal to the time of day, for annual cosinor equal to the date and time of the year), its length indicates the amplitude; the circle (in some cases an ellipse) indicates the confidence interval. If the radius of the confidence interval is greater than the amplitude, the calculated rhythm is not significant for the selected p-value.

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10.1 Data series

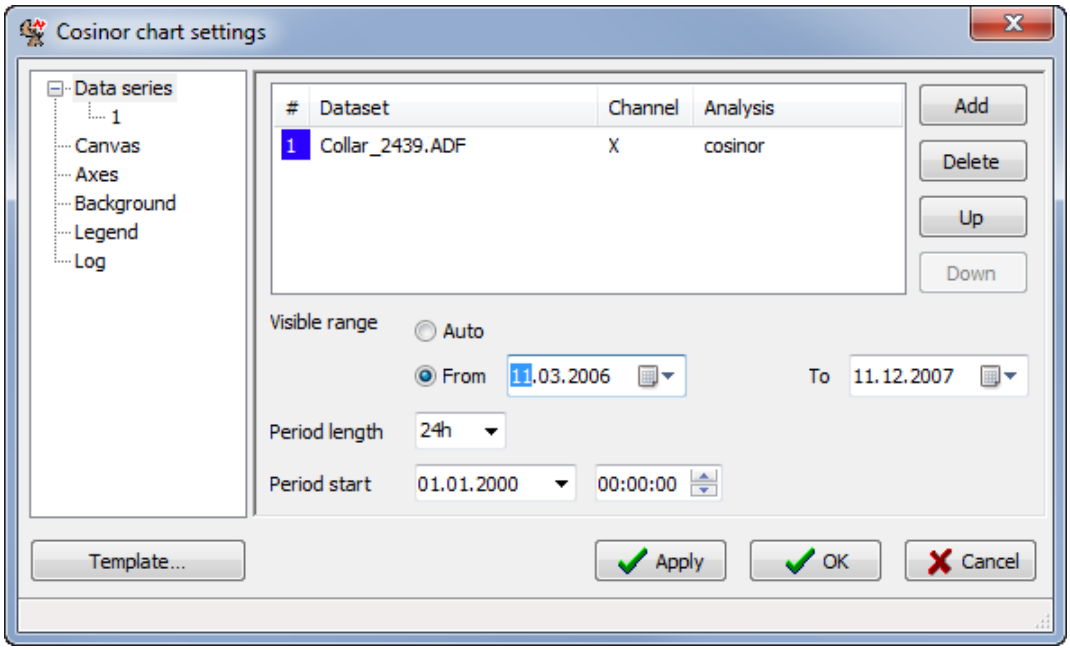

Figure 63: Data series section for cosinor analysis

As described in 7.1, select a previously loaded ADF file for the list with Add or delete an already chosen one with Delete. If you press Add, the window will change automatically to Figure 64.

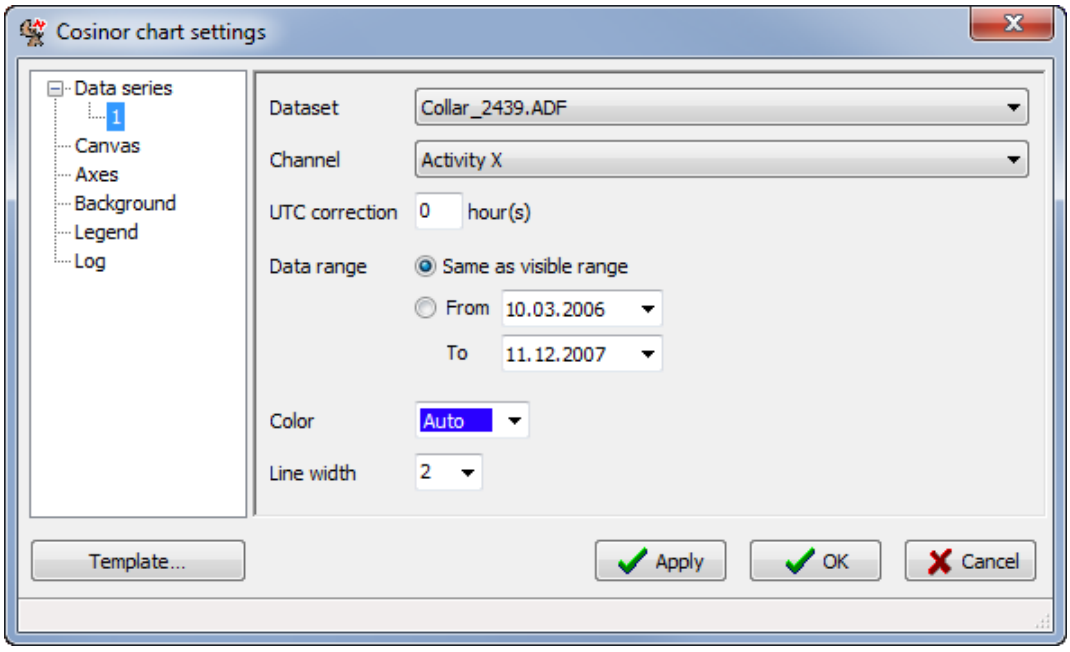

Figure 64: Window for choosing the settings for cosinor analysis

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In the new section, select the *Channel* you want to analyse. In the next line, add the *UTC correction in hours*. For the *Data range*, either check the button *Same as visible range* (default) or check the *From – To* boxes and use the calendar function. You can also choose the *Color* of the vector with the colour box and the width of the line (*Line width*).

After these selections return to the *Data series* section (Figure 63). You will now find the selected file in the list with a running number, the colour of the vector, the analysed channel and the analysis parameter. You can Add new ADF files to the list, Delete files or move them Up or Down in the list.

Choose the time period that will be plotted with *Visible range*. This setting is valid for all files in the list and will override the range selected for a single data series. *Auto* will cover the complete dataset, *From* allows you to choose a data range. The cosinor *Period length* box has presets for 24 hours, seven days, 28 days and one year, but you can also type in any other duration manually. The correct syntax is the number of units followed directly by the letter 'h' for hours, 'd' for days or 'y' for years. To see some examples, take a look at the available presets by clicking the drop-down arrow of the *Period length* box. *Period start specifies* the date and time at which the cosine curve you fit to your data series will begin.

Note: The circle of the cosinor plot resembles one complete period. *Period start* equals 0° of this circle, which is defined as 00:00 when testing a 24-hour rhythm and 00:00 on the 1 of January when testing an annual rhythm. If you manually change *Period start*, this definition will be overruled and the date and time calculated by Activity Pattern will be incorrect. Changing the time of *Period start* is only valid for periods not based on the 24 hours or annual rhythm, e.g. 12 hours or 7 or 28 days. For these periods you have to set the actual start of the cosine curve (the time of *Period start*) manually before you calculate the time or date of the acrophase.

10.2 Canvas

See 7.2.

10.3 Axes

See 7.3.

10.4 Background

See 7.6.

10.5 Legend

See 7.6.

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10.6 Log

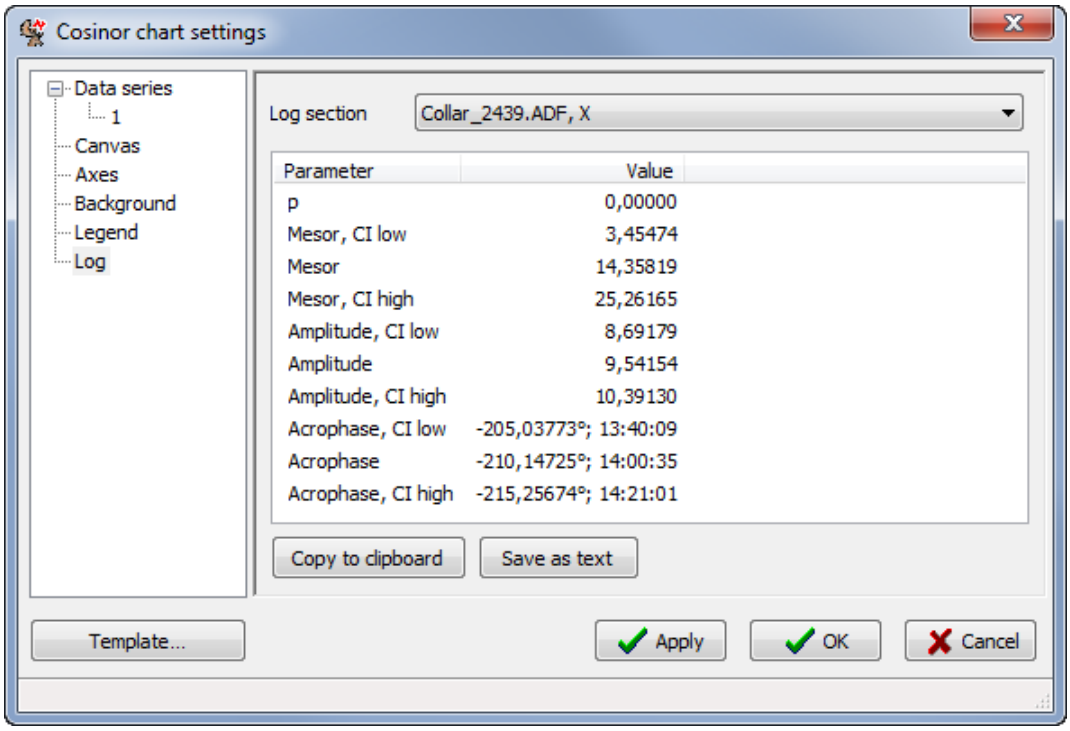

Figure 65: Log section for cosinor, tested period 24 hours

The detailed numerical results of the cosinor analysis can be accessed by the log section. The list includes the confidence level p, as well as the values for mesor, amplitude and acrophase. For these values, the lower and upper boundaries of the confidence interval (*CI low* and *CI high*) are given. If the 24 hour period is tested, the acrophase is given as degree and as time of day. In case of the annual period, the date is given too. For any other tested period, only the degree is given.

Copy to clipboard allows you to insert the data as a table into another program (e.g., MS Office, OpenOffice or a text editor). Save as text saves the data as a tab-delimited text file.

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11 Time Budget

Time Budget (shortcut Ctrl + 6) is a tool to calculate the activity time, the number and the duration of activity peaks based on the activity threshold defined by the user. It also calculates the relation of activity between day and night (Diurnality Index). For details on the analyses and settings refer to Chapters 4-4 Diurnality Index and 4-5 Peak Analysis.

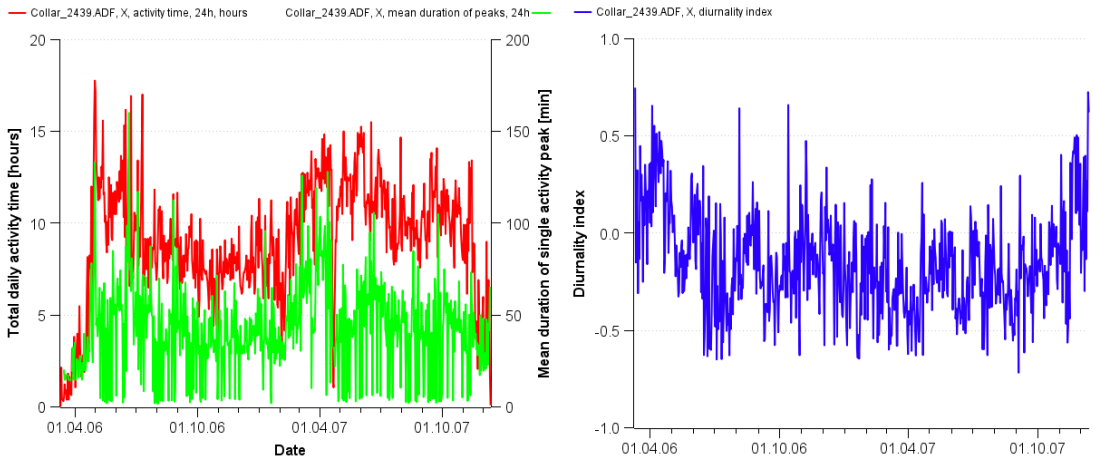

Figure 66: Charts for Time Budget. Left: Total daily activity time (left y-axis) and mean duration of single activity peak (right y-axis). Right: Diurnality index, positive values indicate relatively more activity at daytime, negative values indicate relatively

11.1 Data series

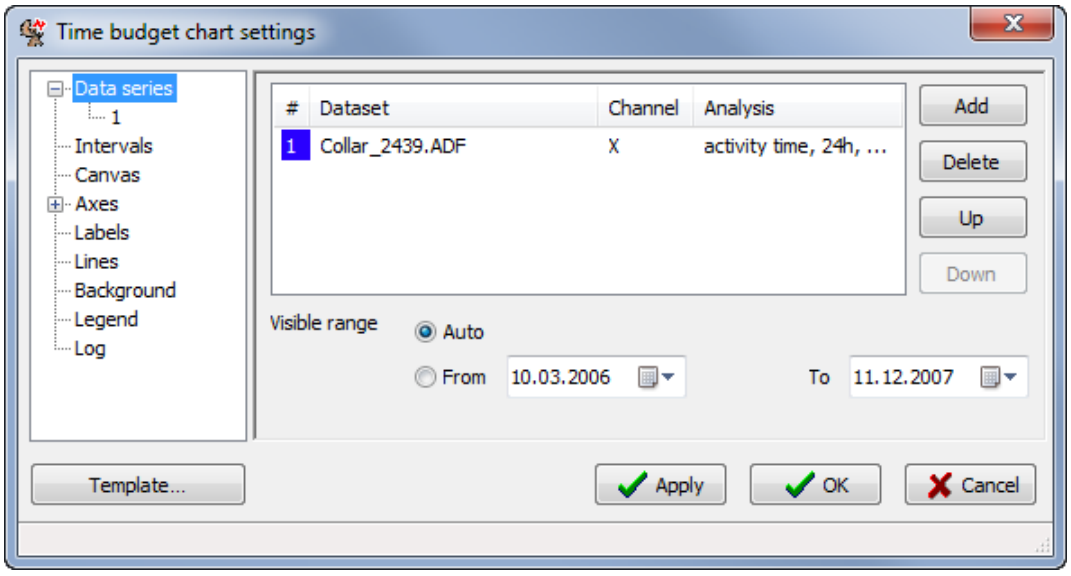

Figure 67: Data series section for time budget

As described in 7.1, select a previously loaded ADF file for the list with Add or delete an already chosen one with Delete. If you press Add, the view will switch automatically to the

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one shown in Figure 68. Select the *Channel* you want to analyse. In the next line, add the *UTC correction in hours*. For the *Data range*, either check the button *Same as visible range* (default) or check the *From – To* boxes and use the calendar function.

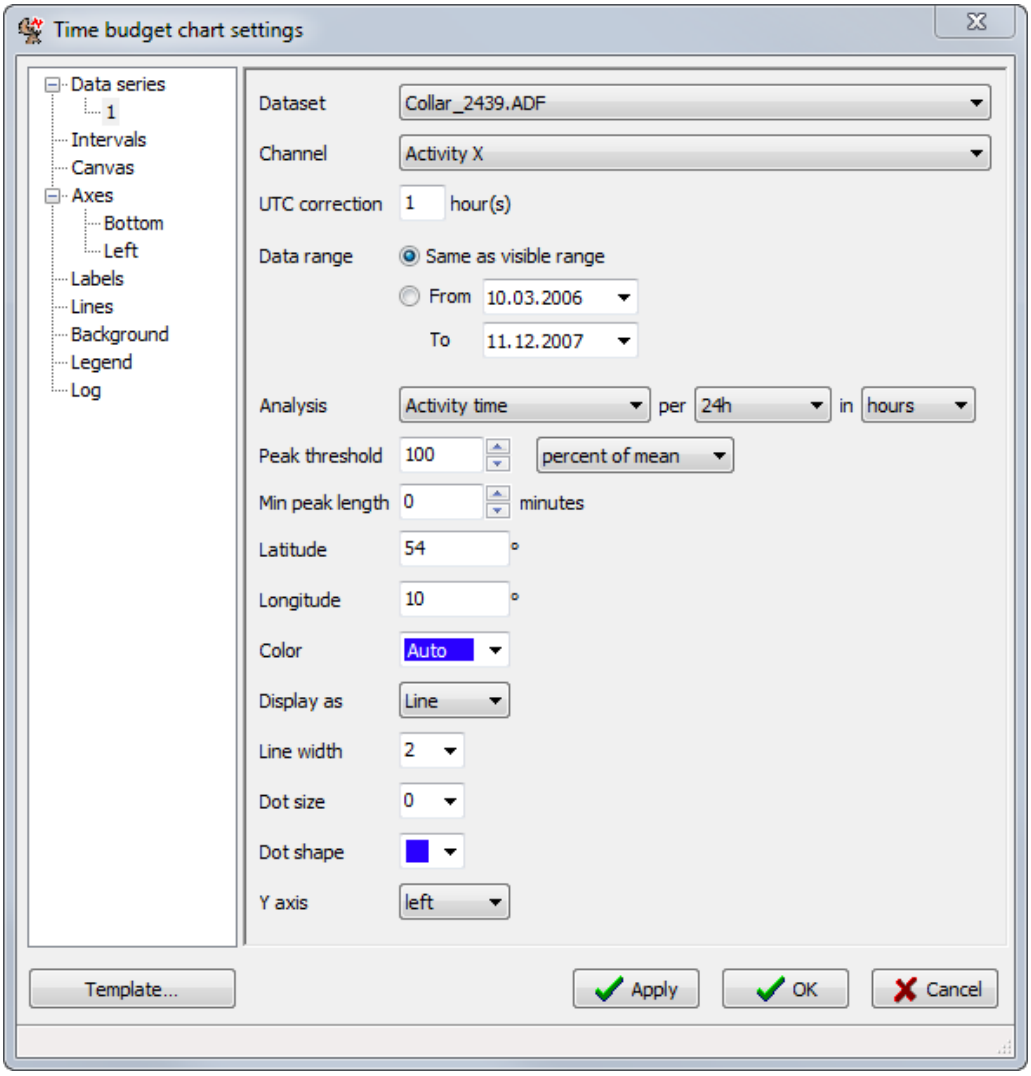

Figure 68: Window for choosing the settings for time budget

In the data series section, select the *Channel* you want to analyse. In the next line, add the *UTC correction in hours*. For the *Data range*, either check the button *Same as visible range* (default) or check the *From – To* boxes and use the calendar function.

With *Analysis*, you can choose between *Activity time*, *Resting time*, *Number of peaks*, *Number of breaks*, *Mean duration of peaks*, *Mean duration of breaks*, and *Diurnality index*. *Activity time* can be calculated for the complete *24 hours* of a day, for the *day* and for the *night*. It is given in *hours* or in *percent*. Number and mean duration of peaks can also be calculated for the complete *24 hours* of a day, for the *day* or for the *night*. If you choose *24 hours*, activity time will be calculated from sunrise of the given day to sunrise of the following day.

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Day and *Night* are intervals which can be defined by the user. By default, *Day* covers the time between sunrise and sunset and *Night* the remaining time. With the *Intervals* section, *Day* and *Night* can be redefined and renamed to custom (see 11.2).

To calculate activity time and peaks, a threshold is needed that allows the distinction between inactive and active values to be made. This threshold will differ between species, individuals, seasons and sensors and therefore needs to be calibrated for every dataset separately. With *Peak threshold*, you can set this value either as an absolute activity value between 0 and 255 (a*bsolute value*) or as a percentage of the mean (*percent of mean*) or median of activity (*percent of median*). The minimal duration of activity peaks can be selected with *Min peak length*. If the activity level exceeds the *Peak threshold* for less than the *Min peak length*, no peak is counted. If the activity level exceeds the *Peak threshold* for more than the *Min peak length*, the peak length is measured from the first interval in which the *Peak threshold* is exceeded. For more details on choosing a suitable peak threshold and length refer to Part II, Chapter 4-5.

Since the calculations in *Time budget* are related to day length, it is necessary to enter the *Latitude* and *Longitude* at which the data have been collected. In the time budget analysis,"24 hours" are defined as sunrise on the given date until next sunrise, to avoid splitting the night.

You can also select the *Color* of the line with the colour box. Use the *Auto* selection, choose between 13 predefined colours or access the colour dialog with the *Custom…* button (Part I, Chapter 4.1.2). With *Display as*, you can choose to display the results as line or as bar chart.

You can also change the width of the line (*Line width*) and select dots to mark the values with *Dot size* and *Dot shape*. The dots will always have the same colour as the line. You can also choose if the *Y axis* for this data series will be on the left or right side of the graph.

After these selections return to the *Data series* section (Figure 67). You will now find the selected file in the list with a running number, the colour of the line, the analysed channel and the analysis parameter. You can Add new ADF files to the list, Delete files or move them Up or Down in the list. Choose the time period that will be plotted with *Visible range*. This setting is valid for all files in the list and will override the range selected for a single data series.

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11.2 Intervals

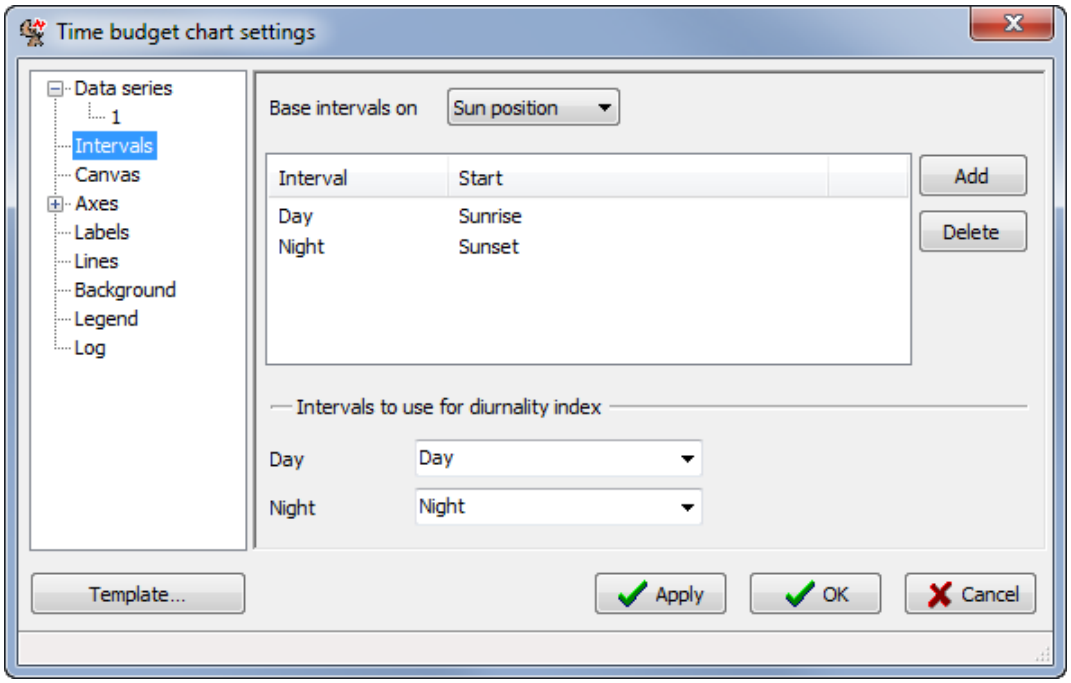

Figure 69: Intervals section

The Diurnality Index can be calculated for any two selected intervals. You can define the intervals with the drop-down menu *Base intervals on* either on the position of the sun (Sun position) or on the Time of day. The defined intervals are shown in the list with the time of their start. Default intervals are *Day* (beginning at sunrise) and *Night* (beginning at sunset). An interval ends at the beginning of the next interval in the list. For calculation of the diurnality index choose two intervals which will be compared with each other. These intervals do not necessarily cover the complete 24 hours of the day. Instead, this feature enables you for example to exclude twilight. You can also compare any other two parts of the day if this is useful for your study. To do this, you need to define new intervals.

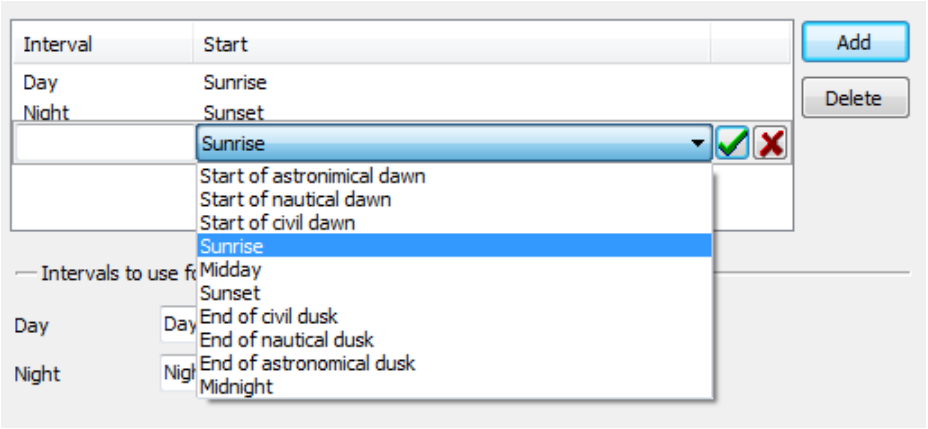

Figure 70: Drop-down menu for defining intervals

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Press Add to define a new interval. A new line will be added with a drop-down menu that allows you to choose the start of the interval. Type the name of the new interval into the left box. Use the green check mark to confirm you selection or the red cross to cancel.

Interval	Start
Dawn	Start of nautical dawn
Day	Sunrise
Dusk	Sunset
Night	End of nautical dusk

Figure 71: List of Intervals (example). Interval Dawn starts with the start of nautical twilight and ends with sunrise, when interval Day starts.

The intervals will be listed in order of their beginning. An interval ends with the start of the next interval in the list. If you calculate the diurnality index for *Dawn* and *Dusk* with the intervals set up as shown in Figure 71, Activity Pattern will use the data between the start of nautical twilight and sunrise for the dawn and the time between sunset and the end of nautical twilight for the dusk. The remaining time is not included in the analysis. If you want to include the complete 24 hours, delete all intervals except for two you want to keep from the list. To do this, highlight an interval and press Delete.

11.3 Axes
See 7.3

11.4 Labels
See 7.4.

11.5 Lines
See 7.5.

11.6 Background
See 7.6.

11.7 Legend
See 7.6.

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11.8 Log

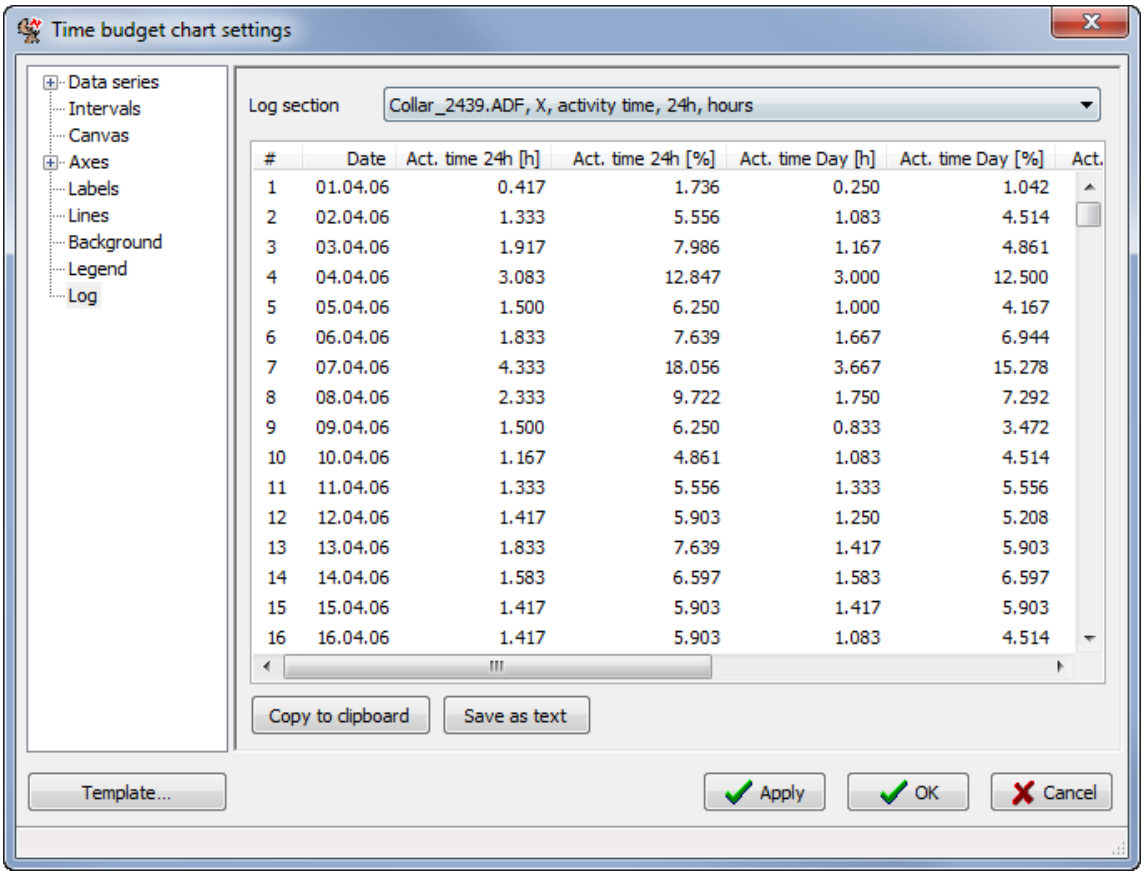

Figure 72: Log section. The detailed numerical results of the time budget analysis can be accessed in the log section. Results are even given calculated for every single day or for the complete data range. This list includes the following parameters (In this example, the default intervals *Day* (interval 1) and *Night* (interval 2) are given. The terms *Day* and *Night* will change if you rename the intervals with the *Interval* section):

- Activity time per 24 hours (*Act. time 24h [h]*)
- Activity time per 24 hours (*Act. time 24h [%]*)
- Activity time during interval 1 (e.g., *Act. time Day [h]*)
- Activity time during the interval 1 (e.g., *Act. time Day [%]*)
- Activity time during the interval 2 (e.g., *Act. time Night [h]*)
- Activity time during the interval 2 (e.g., *Act. time Night [%]*)
- *Diurnality index* (calculated by interval 1 - *Day* - and interval 2 - *Night*)
- Number of peaks per 24 hours (*Num. peaks 24h*)
- Number of peaks during interval 1 (e.g. *Num. peaks day*)

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- Number of peaks during interval 2 (e.g. *Num. peaks night*)
- Mean duration of peaks per 24 hours (*Mean dur. of peaks 24h [min]*)
- Mean duration of peaks during day (*Mean dur. of peaks day [min]*)
- Mean duration of peaks during night (*Mean dur. of peaks night [min]*)

Copy to clipboard allows you to insert the data as a table into another program (e.g., MS Word, MS Excel, OpenOffice or a text editor). Save as text saves the data as a tab-delimited text file.

12 Appendix I: Keyboard shortcuts

[Ctrl + 1] *Charts* → *Actogram*	creates an actogram
[Ctrl + 2] *Charts* → *Statistical by date…*	creates a chart with data organised by date
[Ctrl + 3] *Charts* → *Statistical by time…*	creates a chart with data organised by time of day
[Ctrl + 4] *Charts* → *Rhythm analysis*	creates a chart with Degree of Functional Coupling and Harmonic part
[Ctrl + 5] *Charts* → *Cosinor*	creates a chart with cosinor analysis
[Ctrl + 6] *Charts* → *Time budget*	calculates activity times, diurnality index and activity peaks
[Ctrl + N] *File* → *New workspace*	opens a new workspace
[Ctrl + O] *File* → *Open workspace*	opens an existing workspace
[Ctrl + S] *Save workspace*	saves the opened workspace; option appears after changes have been made to the workspace
[Ctrl + L] *Load ADF file…*	loads an ADF file into the workspace
[Ctrl + D] *Load data from database…*	loads data from your GPS Plus database
[Alt + F4] *Exit*	exits the program

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13 Appendix II: Formats for date and time on actogram and chart axes

h	1-12 hour value without leading zero
hh	1-12 hour value with leading zero
t	AM/PM indicator
H	0-23 hour value without leading zero
HH	0-23 hour value with leading zero
n	whole number of hours without leading zero, may be greater than 23
m	minutes without leading zero
mm	minutes with leading zero
s	seconds without leading zero
ss	seconds with leading zero
f+	floating point number with fractional digits number one less than the number of 'f's
YY	last two digits of the year
YYYY	four digits year string
M	month number without leading zero
MM	month number with leading zero
MMM	month name, short form
MMMM	month name, long form
D	day without leading zero
DD	day with leading zero

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14 Appendix III: ADF Merge: Preparing data for Activity Pattern

Activity Pattern is only able to read ADF files as obtained from your GPS Plus Collar. These files might contain data not from your animal (e.g. test data) or faulty data (e.g. duplicates, wrong time stamp). To edit these data and to clean the file for more convenient and correct analysis in Activity Pattern, use ADF merge.

14.1 Start of program

Copy the file ADFmerge.exe to your computer. You can start the programme with double-clicking on ADFmerge.exe.

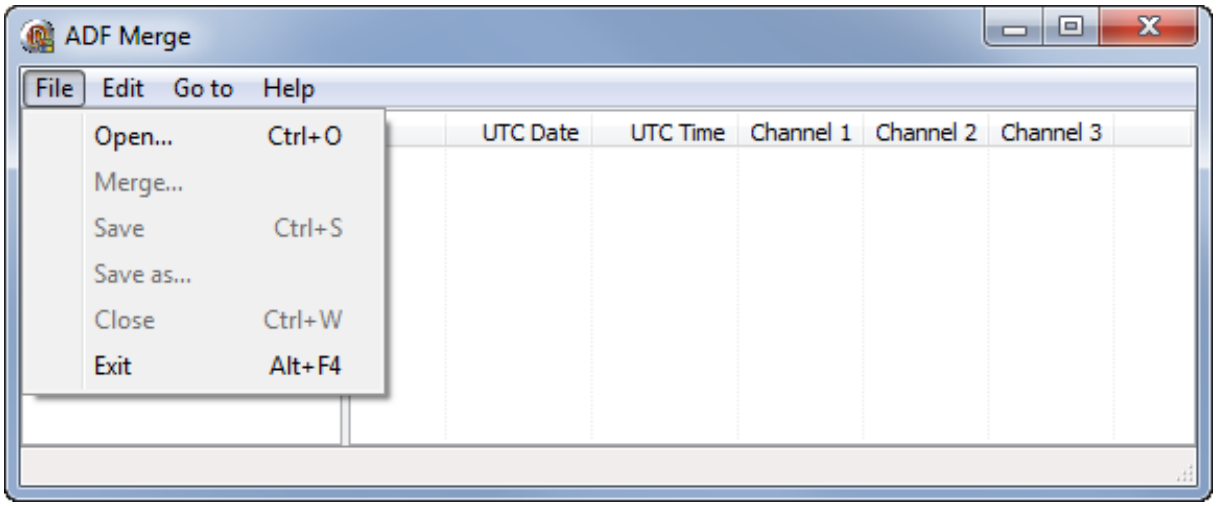

Figure 73: ADF Merge start window

You can open an ADF file with *File → Open...* . The file will immediately be scanned for data gaps and defective samples. The complete data set is now given with a running number, UTC date and time, the measured axes (e.g. X and Y) and the temperature measured by the GPS Plus Collar. Defects are displayed color-coded.

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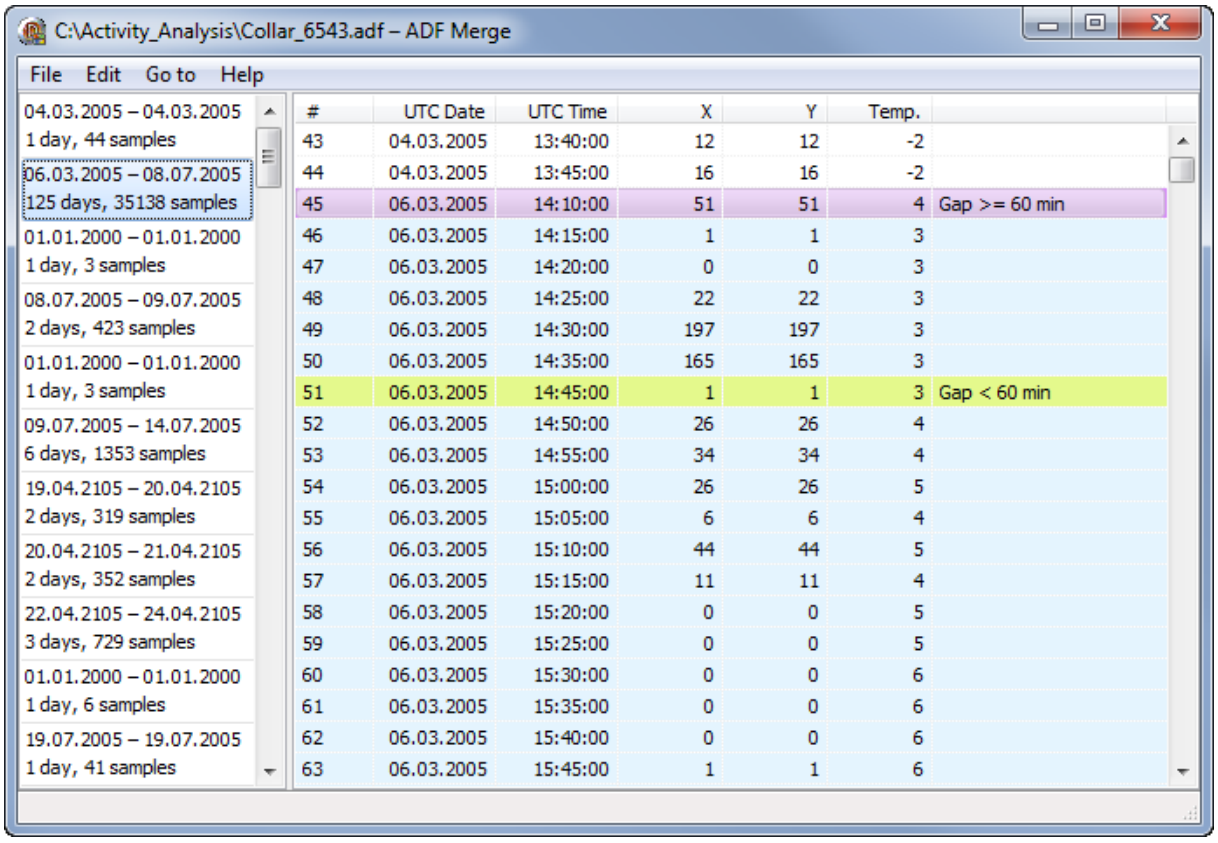

Figure 74: Dataset with sets of continuous data (left) and list of data points with highlighted defects (right)

Four defects are shown:

- Gap > = 60 min
- Gap < 60 min
- Duplicate time stamp
- Negative time offset

14.2 Cleaning data

The left tab displays a list of all continuous datasets with length in days and number of samples. You can jump to the beginning of each dataset by clicking on its box in the left window. The entire dataset is highlighted in blue. To jump between defects use the *Go to* menu respectively Ctrl + Up or Ctrl + Down to jump between defects and Alt + Up or Alt + Down to jump between similar defects.

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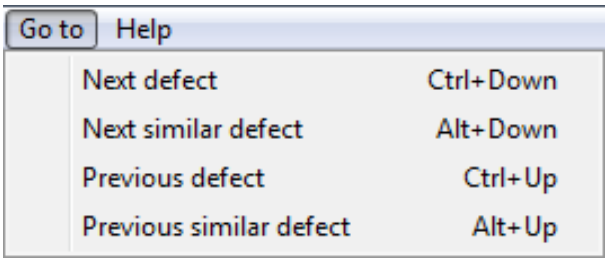

Figure 75: Go to menu

There are three options to clean the data: sort them by timestamp, delete samples and merge two ADF files.

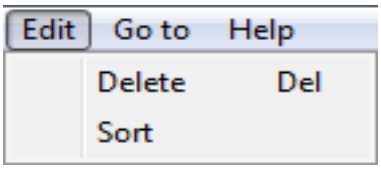

Figure 76: Edit menu

14.3 Sort data

The first step is to sort the samples by timestamp. The samples displayed at the beginning are in order of writing. In some cases it is possible that a wrong timestamp is attached to a dataset, often 01.01.2000 or a date in the future. This is usually combined with a data gap of several intervals, and the data are not safely assignable to a timestamp. It is therefore advisable to delete all datasets with an invalid timestamp. To do this go to *Edit → Sort*. All datasets will now be sorted according to their timestamp. The left window will display a corresponding list of data blocks.

14.4 Delete data

For further analysis it might be useful to delete samples with false timestamps, duplicates and also samples obtained from times during which the collar was not used on the animal (e.g. test data, storage times). You can simply delete samples with the [Delete] key on your keyboard. You can also use *Edit → Delete*. Single samples can be deleted by left-click on the sample in the right window. Sets of continuous data (including false timestamps), can be highlighted in the left window and deleted as block.

Note: You cannot restore deleted data! We strongly advise to keep a backup file of the original ADF.

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14.5 Merge files

After sorting the samples, you can merge two ADF files. To do this, open the first ADF file and go to *File → Merge...* . An opening dialog will appear. Open the second ADF file, which will be attached to the first.

It is possible that timestamps of both ADF files overlap. ADF Merge will display these overlaps in a separate window. Verify the cause of the overlap (e.g. files not from the same animal/collar, files have already been merged) and check-mark the samples you want to keep. Choose OK to discard the unmarked data and merge the remaining samples of the two files.

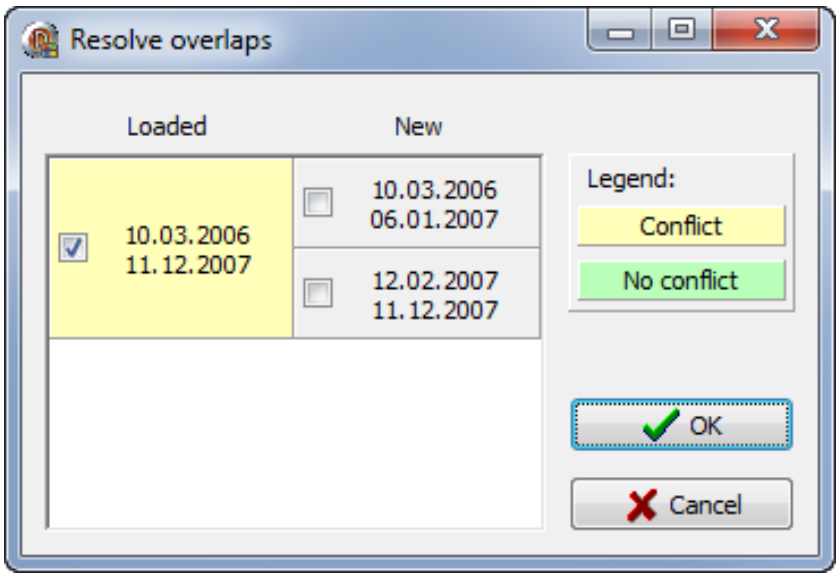

Figure 77: List of overlapping time periods in two ADF files to be merged

Note: Overlapping data should only occur in the case of data defects or if you use two files you have already merged. Before discarding data make sure the files are really from the same animal and that the discarded data are defect. It is advisable to keep a backup of the original ADF!

Note: It is not possible to merge data recorded with different activity modes.

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Part Two:

Theoretical Introduction to Biological Rhythms and Rhythm Analysis

Chapter 1: Introduction

1 What is activity?

It is difficult to give a straight-forward definition for activity. The Oxford Dictionary explains activity as “a condition in which things are happening or being done”. Another, more zoological, explanation would be that activity is everything an animal does, usually defined with exception of sleeping and resting.

This general explanation is not sufficient for a study on activity. In research, the meaning of activity depends on the method or device of measurement. In early pedometers, activity was measured by a lead ball moving back and forth or a pendulum. Today, this has been substituted by electrical switches. This method measures locomotor activity, but to a certain degree also other movements of the body.

Most studies on activity so far have been done in laboratories, usually on small animals. Motion detectors can be employed, but often “activity” is measured by certain behaviors; some of the best studied “activities” is probably wheel running in golden hamsters or hopping between perches in birds. Grooming or social activities are often not measured in these studies. Since there is no general explanation for activity it needs to be defined for every study or at least for every method used.

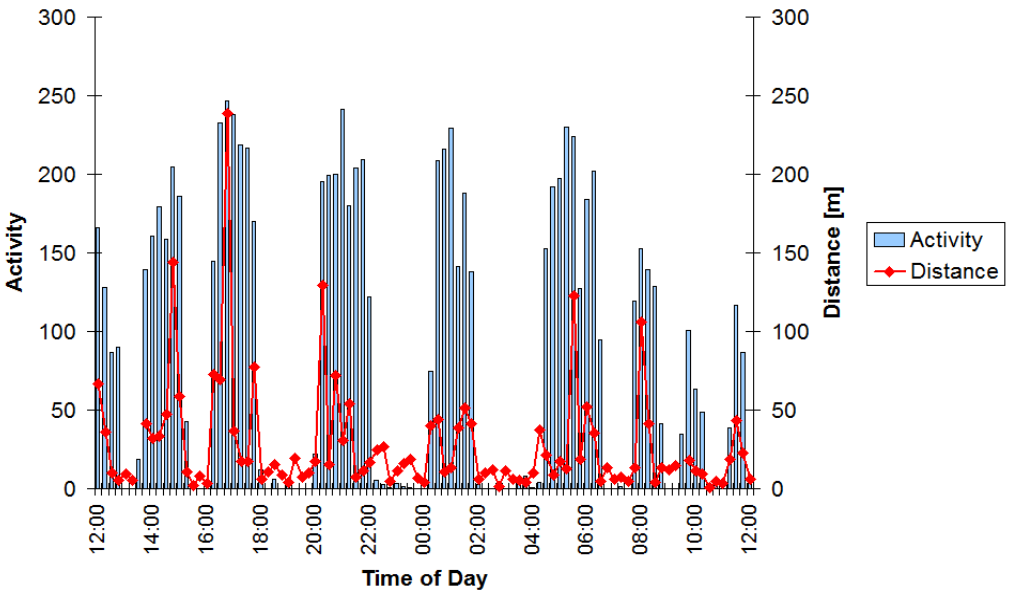

Figure 78: Activity level (grey bars) and distance (red line) between two GPS positionings for a female roe deer over 24 hours measured in 15 minute intervals (measured by the GPS Plus Collar)

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Some definitions of activity are more useful than others, and the research topic needs to be considered. For free-ranging animals, trajectories are most commonly used to estimate locomotor activity. However, this covers only a fraction of the actual locomotor activity, for movements on a small scale are lost. The same is the case with activity not related to locomotion, but to feeding, comfort or social behavior. Figure 78 shows the mean activity level within 15 minutes and the distance covered within the same interval in a female roe deer. It is obvious that though activity and distance correlate, activity is not necessarily low if no distances have been covered due to activity includes a lot of behaviors (like feeding, grooming, social interaction etc.) which have little relation to the change of place.

Another problem in activity measurement is a meaningful measuring technique on free ranging animals. Some telemetric systems discriminate between active and inactive, but do not give information on the intensity of activity. To calculate correct time budgets or energy turnover rates, it is necessary to include all kinds of activity and take the intensity of activity into account, which is achieved with an acceleration sensor.

An acceleration sensor gives you a relative level of movement – acceleration - the sensor has experienced within the sampling interval. This is not a distinct information on the behavior performed by the animal, but using preliminary parallel observations of the species-specific behavior motions and durations and coupling activity to GPS positionings, you can draw conclusions if the animal is fast moving, slow moving or resting (for example see Löttker *et al.* 2009).

2 The ecological value of activity rhythms

We live in a cyclic environment. The most prominent environmental rhythm is the solar cycle, which includes predictable periodic changes in light intensity, temperature and UV radiation. It is no surprise that all organisms exposed to this cycle show periodic changes in many body parameters related to the solar cycle. Although some animal species display regular activity periods during the complete 24 hours of a day, we commonly classify animals as diurnal or nocturnal, and in some cases as crepuscular, meaning active at twilight. In some species, activity patterns are not as easily classified and might appear as random series of activity and resting.

The properties of day and night place the same restraints and demands on an organism as does the habitat. Therefore, Roenneberg (1993) suggests the term “chronotope” as temporal equivalent for habitat or biotope. A big difference between habitat and chronotope is the fact that the habitat is rather constant or changes over a long period of time while the chronotope is regularly changing on a small timescale. Important body functions have to be adapted to this change.

The origin of biological rhythms is believed to be the need in bacteria and protozoa to avoid UV-radiation prior to the existence of the ozone layer. Organisms capable of confining their activity to the night had a selective advantage. Today, organisms inhabit all chronotopes.

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They are well adapted to the part of the solar cycle they are active in and cannot change this easily. The extend of this adaptation becomes clearer if you keep in mind that complete phylogenetic groups have developed the same activity times. Mammals have originally evolved as nocturnal animals to avoid predation by reptiles and dinosaurs. This was possible, since mammal thermoregulation became independent from external heat sources like the sun while reptiles were restricted in their nocturnal activity. The cretaceous mammals relied mainly on olfactory and acoustic senses. Only at a later point of mammal evolution, diurnal species with highly evolved visual senses emerged. Today, mammal species can be found in all chronotopes. Birds, on the other hand, primarily evolved as diurnal organisms with visual reception of their environment; nocturnal birds are in minority.

The adaptation to the chronotope does not only include evolutionary developed anatomy, most physiological parameters are involved too. Blood pressure, respiratory volume, body temperature, concentration of electrolytes, hormones (e.g. cortisol) and blood cells as well as activity of gut, stomach, liver and kidneys have been shown to display several daily rhythms which are related to the physiological resting or activity times. Times of birth and egg disposition, begin of oestrus and time of death displays concentrations at certain times of day and year too. These evolutionary adaptations in physiology ensure high performance during physiological activity times and enable the animal to save energy by putting the organism in a "stand-by-mode" during resting times.

Why is the right timing so important? Being active at the wrong time might mean that an animal encounters temperatures to high or low for its physiology. Getting up to late shortens an animal's time for exploration and hunting, getting up to early wastes energy. Time-place learning, especially the knowledge at which time of the day food is found in which places, is common in several species; its endogenous control has been demonstrated for garden warblers (Biebach *et al.* 1991). Daily and seasonal changes in environment restrict windows of opportunity in time for animal activities and, hence, the question of when to do what is subject to fitness maximization and the task of structuring several activities is two-sided: It consists of the total amount of time (the time budget) and its temporal order of sequences (the time flow).

For social animals, the intraspecific coordination of activity times is vital for many aspects of their life, including safety in numbers from predators, protection of territory from competitors, and acquisition of mates or prey. Coordination is also important on the interspecific level. Predators orient their activity times to that of their prey to increase hunting success. For species using the same resources, it might be useful to separate the time of activity. This has led to the evolution of diurnal and nocturnal species using the same habitat: by adapting to the day or night chronotope, two species can use the same resources without direct competition.

Very impressive are the results on free-ranging chipmunks (*Tamias striatus*). Individuals which due to brain surgery did not display the species-specific diurnal activity pattern, but were also active at night, had a higher risk of being killed by weasel predation than those displaying a diurnal pattern (DeCoursey *et al.* 2000). This is a good example for the

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importance of a temporal niche to avoid predators living in the same area, but with a different activity time or temporal niche.

In fact, we find temporal patterns related to the daily periodicity of the environment on almost every level of life, from cell to communities as well as biotic factors of an individual reveals periodic features. Conspecifics, competitors, parasites, prey and predators all have their own time schedule, and the synchronisation of these schedules is essential for fitness maximization. This synchronisation is done by an internal timing system, the "Internal Clock". We will focus on the properties and relevance of the internal clock and activity patterns in Chapter 2.

3 Applied activity rhythm research – some ideas for the researcher

Modern wildlife collars are commonly used to obtain regular GPS locations which give information on home range size and use, migration paths, etc. Unfortunately, GPS fixes need a considerable amount of energy, so in most studies, only few GPS fixes per day are obtained. This results in a low number and frequency of data. Activity measurements on the contrary need little energy, thus allowing a virtually continuous monitoring of an animal. This enables you to study activity patterns in free-ranging animals in an extent that has not been possible before. But what is the use of such a measurement?

3.1 Activity Pattern research in the field

There have been several breakthroughs on time-organization on genetic or organismic level, and activity has been studied extensively in the laboratory, thereas little is known about activity rhythms under field conditions. In the controlled conditions of captivity, activity patterns are regular and predictable. In the field, animals need to cope with a variety of external aspects which might influence the pattern. The golden hamster, *Mesocricetus auratus*, for example is well known for being a nocturnal animal with a very reliable activity pattern; it has been widely used to for basic research on biological rhythms. Recent field studies now revealed that wild golden hamsters display a variety of activity rhythms covering the complete 24 hours, not only the day (Gattermann *et al.* 2008).

Within a species, activity patterns can differ between sexes, age classes or populations living in different habitats and climates. There are also seasonal changes within individuals. Food restrictions (e.g. in winter) often lead to a decrease in activity, and rut or migration can result in not only higher activity, but very different daily patterns too. There are also days in which special events take place which have an impact on activity as mating, calving or human hunt; detailed analysis of activity patterns might reveal these events.

Changes in habitat can have severe influences on the activity patterns and give information on coping mechanisms of a species. Today, humans have a high impact on wild animals and habitats often change rapidly under human influence. A topic regularly discussed is the

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influence of hunting pressure or human presence in general which has been shown to shift activity of several species into the night (for review see Scheibe 2009). The loss or re-introduction of predators has been shown to alter alertness and activity patterns in ungulates (Laundré *et al.* 2001). Changes in vegetation can also influence activity patterns, since deforestation amongst other things changes the photoperiod and light intensity experienced by animals living on forest ground level (DeCorsey 1961). Since weather parameter have been shown to influence activity patterns in some species (e.g. Beier and McCullough 1990), climate change is most likely to have an impact on activity patterns.

Since activity and its timing are vital for the fitness of a species, the adaptability to new conditions is important for its survival. The extent of this flexibility depends on the species. Some species are highly adapted to certain activity times and display rigid activity rhythms. Others are more opportunistic and vary their activity times according to changes in the habitat. Thus, the knowledge of activity times and the understanding of their adaptability is an important part of today's efforts in conservation and must be considered in management plans.

3.2 Habitat use – GPS plus activity sensor

Spatial use is an important aspect in a species' ecology. GPS fixes provide valuable information on the actual places used by the animals, which can be correlated to information on which kind of habitat is used. GPS fixes have relatively high energy demands, which is the reason for the usually high intervals between fixes. Activity on the other side is measured continuously and can be stored in intervals as short as 64 seconds. This provides a virtually constant monitoring of an animal's level of activity, and this for a long time span without exploiting the battery.

Another tempting approach is the combination of GPS location and activity. GPS on its own provides only limited information on the actual use of a location. A high number of different locations in a short time suggest activity in an animal, but the animal might have changed places directly and spent the remaining time resting. Then again, an animal might be active over a longer time span in one place, which is often the case in grazing animals (see Part II, Chapter 2-1). Therefore, measuring activity is a valuable addition to GPS. The information on the level of activity in a certain location also allows conclusion about the function of a place. First approaches to discriminate resting, slow and fast locomotion in red deer based on the acceleration measurements have been promising (Löttker *et al.* 2009). This might identify resting places or locations with high activity behaviors, e.g. migration routes.

3.3 Monitoring animal well-being

In the last years, animal well-being has become important for any kind of animal husbandry and wildlife management. Major requests are adequate housing and the protection from unnecessary suffering. A number of methods and criteria for the assessment of well-being have been discussed, but none seems to be satisfactory on its own. The discussion begins with a valid definition of well-being and the question which criteria have to be considered.

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Stress is a term often used in combination with animal welfare, but is not necessarily clearly understood. Selye (1950) defined stress as increased activity of the hypothalamic-pituitary adrenal axis, a definition still valid today. It is also well-known that stress response patterns involve more parameters, one of these can be activity.

Another problem is to distinguish eustress from distress by simply relying on hormonal data without a behavioral evaluation. Stallions during mating for example have high levels of stress hormones, but this is not necessarily an impairment of their well-being (Colborn *et al.* 1991). On the other hand, in animals displaying stereotypical behavior levels of stress hormones are often surprisingly low, for stereotypical behavior has shown to releases anti-stress hormones like serotonine (Broom 1988).

Further problem of using physiological parameters for the assessment of well-being is the often invasive method of obtaining these data. Capturing animals to take blood samples can influence their stress hormone levels and thus lead to false results. Its information is also restricted to the moment the sample is taken, and thus not a long-term assessment. Faecal samples have been established to assess stress non-invasively on a long-term basis, but samples have to be fresh and individual identification is not always possible. For both methods, laboratory processing is needed, which can lead to a time-lag in obtaining the results and high costs too.

Activity can be a useful parameter to assess well-being in animals. It has been shown under seminatural and captive conditions that the rhythmic pattern of activity changes with stress, disease or other disturbances (Berger *et al.* 1998; Berger *et al.* 2003; Scheibe *et al.* 1978; Scheibe *et al.* 1995; Scheibe *et al.* 1999). These changes might be severe and easily detectable, or they might be subtle and only recognizable using rhythm analysis as described in Part II, Chapter 4-7. The use of activity patterns for assessing well-being is a research topic not widely established by now, but it provides a number of questions to answer and hence good possibilities for further research.

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Chapter 2: A theoretical introduction into rhythm research

In addition to the traditional methods of activity analysis, Activity Pattern is able to analyse activity rhythms. This chapter will give you an introduction into biological rhythms. However, for proper studies of this subject we advise you to read the literature suggested in this chapter.

1 Biological rhythms

1.1 The properties of biological rhythms

The scientific observation of cyclic biological parameters is no new research topic. A well known example from the 18th century is the flower clock by Carolus Linnaeus, consisting of different plants that open their flowers at specific times of day. Today, biological rhythms are widely studied in medicine and pharmacology.

For animal research, activity is probably one of the parameters most easily measured. Daily rhythms of locomotor activity have been studied as early as the 1920s and until today there has been a variety of studies on small mammals and birds, though mostly in laboratories. These experiments have provided profound basic knowledge to understand the basic characteristics of biological rhythms.

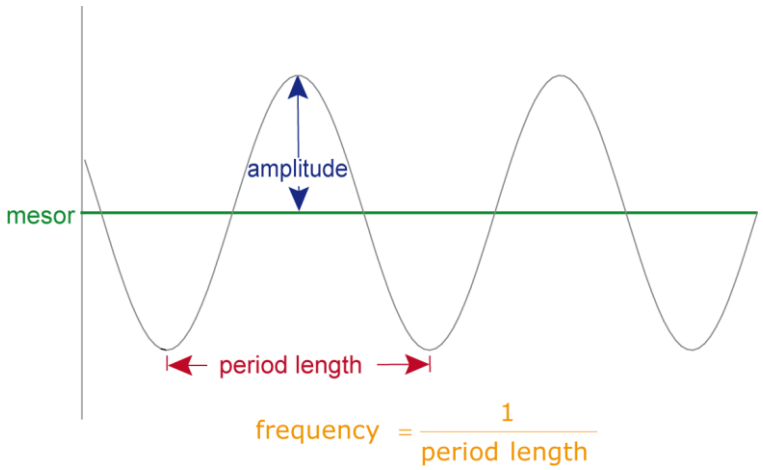

Figure 79: Diagram of an oscillation

Biological rhythms are oscillations and are thus characterized by period length τ, frequency *f* ($f = 1/\tau$), amplitude and mesor (Figure 79). The period length is the time elapsed for one complete oscillation or, in other words, the time between two peaks; frequency is the reciprocal of the period *f* ($f = 1/\tau$). The amplitude represents the difference between the peak and the mean or mesor. MESOR (abbr. for Midline Estimating Statistic Of Rhythm) is a rhythm adjusted mean. If a process is known to be rhythmic, but data points are not

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equidistant or sample size is small, the arithmetic mean may be biased, if for example most of the data points have been sampled around the peaks. The mesor adjusts for this, and in equidistant data its standard error is usually smaller. There has been a substantial interest whether measured periodic events are externally or endogenously controlled. Internally controlled rhythms persist even in constant light-dark conditions, but not with the former period length τ (mostly 24 hours), but with a slightly longer or shorter one. This results in an onset of activity at a different time of each day, but the time lag between following days is constant. We now speak of a free-running rhythm (Figure 80).

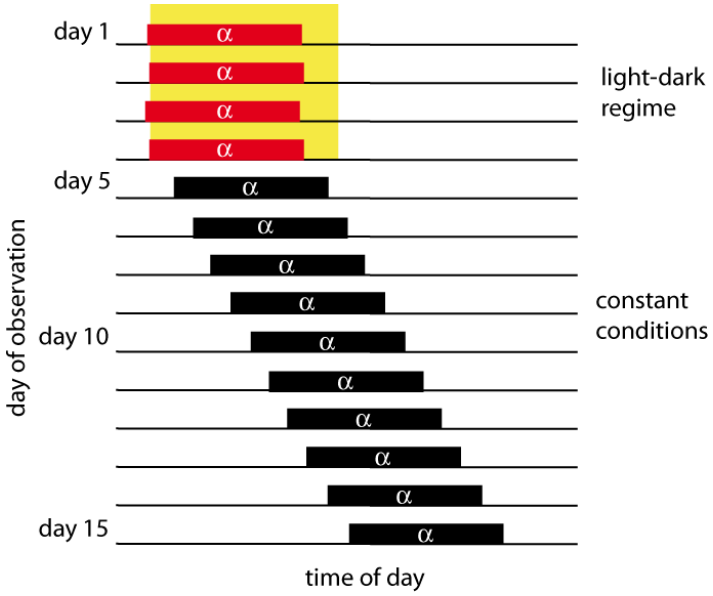

Figure 80: Example for a free-running rhythm in a diurnal animal. Yellow = light, α = time of activity, red = rhythm with regular onset of activity, black = free-running rhythm.

The free-running period length of daily rhythms is very rigid and shows little variations between single periods. Depending on the species and the parameter (activity, body temperature, etc.) the period length of daily rhythms lies in a range 22 and 28 hours. Due to the period lengths of not exactly 24 hours or one day, endogenous daily rhythm are called circadian rhythms (latin for circa = about and dia = day). As a general rule, period lengths in nocturnal species are shorter than 24 hours, in diurnal species longer than 24 hours. The exactness of the circadian period length is amazing: in rodents the variation within individuals is around 1-2 min, while interindividual differences within one species mostly up to 1 hour or more. The free-running period length is independent from temperature. It is obvious that if the correct timing is vital for survival, temperature changes in the environment must not influence the period length.

True biological rhythms are genetically determined and inherent. Even if an individual has not experienced a rhythmic environment as embryo, biological rhythms are displayed later in life. Biological rhythms are not restricted to daily rhythms; there is a general discrimination in ultradian ($\tau < 24$ h), circadian (~ 24 hours) and infradian ($\tau > 24$ h) rhythms. Another group well investigated are the seasonal or circannual rhythms, however, due to their lengths it is

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difficult to study them under constant conditions. Less well known are the properties of circatidal and circalunar rhythms, which are especially important for marine organisms. For more details on the different rhythms see Part II, Chapter 2-3).

1.2 Zeitgeber

If the period length of circadian rhythms under constant conditions differs from 24 hours, but under natural conditions is similar to the environmental periodicity, there must be a way to synchronise endogenous rhythms with the environment. This is done by external time cues or *zeitgebers* (German for *Zeit* = time and *Geber* = giver).

To act as a zeitgeber, an external stimulus must display a reliable periodicity and needs to be relevant for an organism. In this case, the zeitgeber stimulus entrains the organism to the external cycle. The most potential – and best studied – zeitgeber is light. Temperature is usually coupled to light and can also act as zeitgeber, especially if light is absent, e.g. in caves or blind animals. It is a stronger zeitgeber in heterothermic species than in homothermic.

In absence of light many rhythmically appearing events can act as zeitgeber and some have been proven to do so. One example is food if it is only available for a certain time of day. Anticipatory behavior prior to feeding times is well known in animal husbandry. Periodic noise can also entrain an animal, especially conspecifics noises in social animals. The importance of social zeitgebers (presence of potential mating partners or group members) has often been discussed but not clearly shown yet. But at least in some species there is a possibility of entrainment between two individuals. More evidence has been demonstrated for the ability of induced activity as zeitgeber.

Although it is generally accepted that the periodic changes of zeitgeber intensity are important for the internal clock, there is still a discussion which part of the change is the actual stimulus. It is possible, that the presence of the zeitgeber (e.g. light) is important; in this case it would be a proportional effect. However, this would not account for free-running rhythms under constant conditions. Instead, the changing of the factors (e.g. the change from light to dark) might be the actual stimulus, in which case it would be a differential effect. It has been demonstrated that twilight widens the entrainment in hamsters (Boulos et al. 1996; Boulos et al. 2002) and that scorpions are entrained more precisely and with a better internal synchrony by low light intensities (Fleissner and Fleissner 1998).

A zeitgeber does not trigger activity – or periodic physiological parameters – in a direct way. As Figure 81 shows, the zeitgeber, e.g. changes in light intensity, are registered by a receptor, in this case the retina. The information is transferred to a pacemaker, a neural structure that generates the circadian rhythm. In this sense, the circadian clock system may be compared to a watch with a spring (pacemaker) that drives it slightly too fast or too slow while it is corrected daily by a person (zeitgeber).

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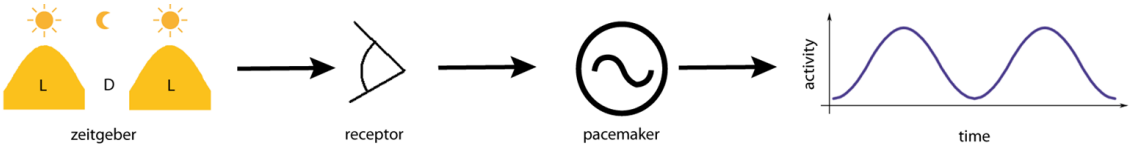

Figure 81: Path of the zeitgeber light

Due to this cascade, there often is a time lag between the appearance of the zeitgeber and the measureable reaction of the organism, e.g. the onset of activity. However, if the zeitgeber disappears, the entrained rhythm remained at least for a few periods, before it returns to the endogenous free-running period length.

1.3 The relation between zeitgeber and endogenous rhythms

Zeitgebers, too, are oscillations, so the relationship between zeitgeber and endogenous rhythm is the relationship of two oscillations. Due to synchronisation by the zeitgeber the period length of both oscillations is similar, but there is a time lapse between the peak of the zeitgeber signal (e.g. sunrise) and the peak of the endogenous rhythm. This time lapse is called phase or phase angle φ and can be given in radians, degrees or other fractions of the whole period, e.g. in time units, if period length is given (Figure 82).

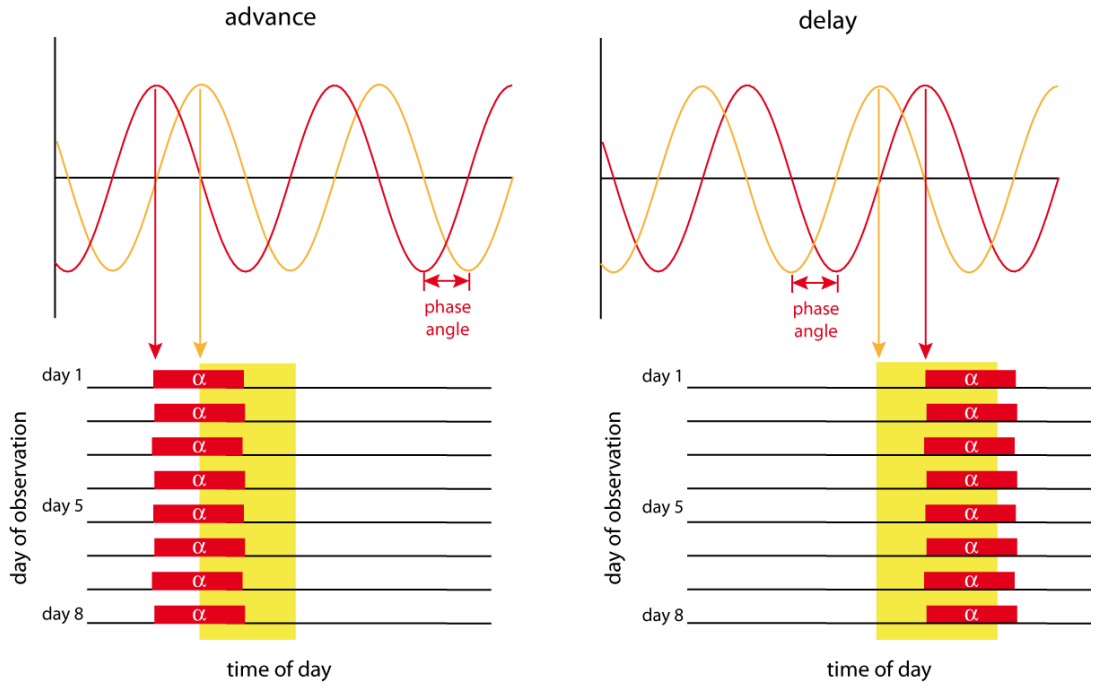

Figure 82: Advanced and delayed phase angles. Yellow = zeitgeber signal light, red = activity

The phase of a biological rhythm depends on at least three parameters of a zeitgeber: the light:dark (L:D) ratio, the L:D frequency and the intensity of illumination in L and D. In diurnal animals, the phase stays more or less parallel to “light-on”, in nocturnal animals parallel to “dark-on”. If the organism’s reaction takes place after the zeitgeber stimulus, the

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phase angle difference is known as “delay” and would be described as a phase with a positive sign. If the organism’s reaction takes place prior to the zeitgeber stimulus, which would be anticipatory behavior, it is called “advance”, a phase with a negative sign (Figure 82).

Changes in zeitgeber timing result in phase shifts, which are single displacements of the phase on the time axis. In this case, a characteristic property of a zeitgeber becomes obvious. Often the phase does not follow the complete shift of the zeitgeber, but needs several periods to resynchronise. The oscillations between the two steady-state oscillations are called transients (Figure 83). This phenomenon is known in humans as jet lag. If the shift for instance is 12 hours, there usually is an immediate exogenous adaptation of activity. However, other physiological parameters of the organism still need time to synchronise and will display transients.

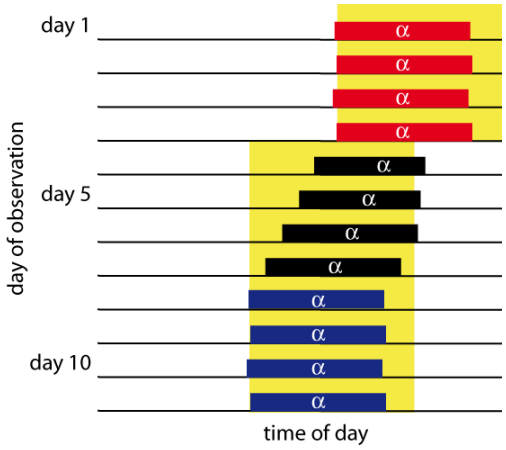

Figure 83: Example for transients in a diurnal animal. Yellow = light, α = time of activity, red = steady-state oscillation prior to shift, black = transients immediately after shift, blue = steady-state oscillation after shift.

The time between shift and resynchronisation depends on the zeitgeber strength and intensity as well as on the general coupling between organism and zeitgeber. In strictly nocturnal species, light is a stronger synchronisation agent than in more flexible species. Additionally, external stimuli like food availability, social partners and induced activity can speed up the resynchronisation.

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1.4 Masking

While zeitgebers affect the pacemaker itself and therefore influence the endogenous rhythm on a basic, long-lasting scale to prepare for periodical changes, animals are confronted daily with less predictable short-term events, e.g. changes in weather, the appearance of a predator or the availability of prey. These events require an immediate answer (e.g. decreased locomotor activity while hiding from a predator), but they must not alter the overt activity rhythm (in this case shifting the resting period into the physiological activity time).

The process of an external stimulus instantly changing an activity pattern is called masking. Masking does not influence the pacemaker and entrain the endogenous rhythm, but directly enhances or inhibits the rhythm without changing its phase or period length. The effect of a masking agent is immediate, in contrast to a zeitgeber shift there are no transients. As soon as the masking agent is withdrawn, the organism returns to the overt rhythm (Figure 84). Masking also never involves anticipatory behavior; anticipatory behavior is set off by the pacemaker and caused by an advanced phase angle between zeitgeber and endogenous rhythm. Due to their short-term influence, masking agents can only be followed by a reaction.

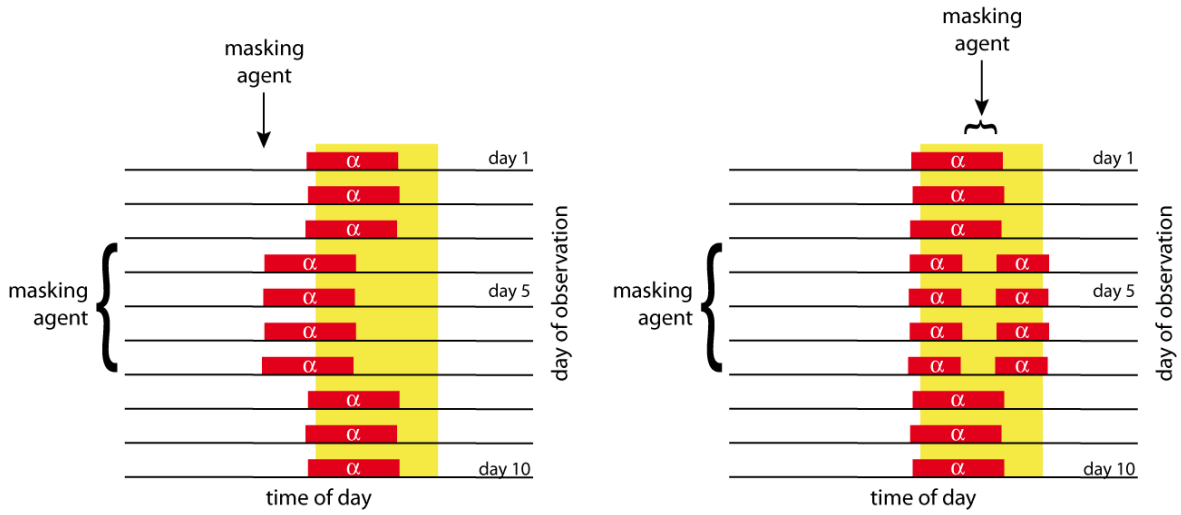

Figure 84: Two simple examples of periodic masking. Left: Activity is immediately induced by the masking agent (e.g. social partner) but returns instantly to the phase angle with the zeitgeber when the masking agent is removed. In contrast to a zeitgeber, there are no transients between both states. Right: Activity during the physiological activity time is suppressed while the masking agent (e.g. predator) is present and resumes after it is removed. The original rhythm is displayed when the masking agent does not appear anymore.

Most laboratory studies try to avoid any kind of masking to concentrate on the overt rhythm, but masking is an important adaptation of the organism to react immediately directly and in an appropriate way to a disturbance. This might be the reason why activity rhythms of the same species differ between laboratory and nature as has been shown by Gattermann et al. (2008) for Golden hamsters. These are mostly nocturnal and display a strict onset of activity under controlled lab conditions, but display a less strict diurnal activity pattern in nature. The complex pattern arising from the combination of entrainment and masking in nature presents a

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challenge for field research, but it is necessary to examine these processes for a thorough understanding of the ecology of biological rhythm and their plasticity.

1.5 The multi-oscillator system

So far we have focused on only one oscillation or parameter, but in an organism, a high number of biological rhythms or oscillations run with different period lengths. Under natural, undisturbed conditions, these rhythms are synchronised by the pacemaker, which means the pacemaker resets the phase of the rhythm to the appropriate phase angle with the zeitgeber oscillation (Figure 85). This synchronisation is done by the pacemaker, which transfers the zeitgeber signal on secondary oscillations. This synchronisation is done every time the zeitgeber appears or reaches a certain value (e.g. light intensity at sunrise).

The relationship between the pacemaker and the inferior oscillations not single-sided. Though the pacemaker transfers the signal on the other oscillations, they can also influence the pacemaker, changing its oscillation too; there are also linked to each other. Due to a reciprocal influence between each other, a complicated pattern arises. In a weak coupling between the inferior oscillations), all involved oscillations interfere with each other and add up to a complicated pattern (“relative coordination. In a strong coupling (between pacemaker and inferior oscillators), a common mean rhythm will result (“absolute coordination which is close to the main oscillation.

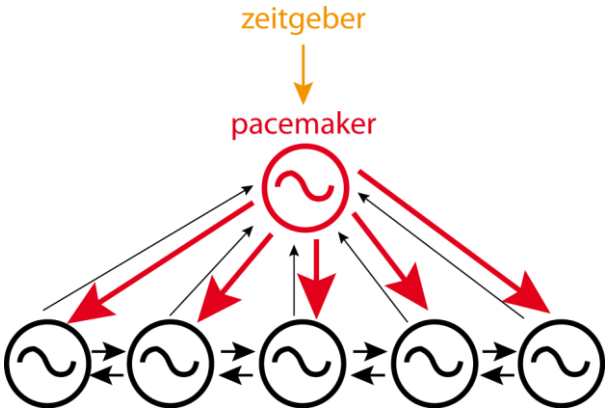

Figure 85: Schema of a multi-oscillator system. The pacemaker is superior to the other oscillators and enforces its oscillation on them. However, due to their coupling with each other, the inferior oscillators influence the pacemaker too, but their influence is weaker. On the second level, the oscillators are coupled to and therefore influence each other.

In a system containing of more than one oscillation, another phenomenon might appear, the correlation. In this case, there is dependence between two or more oscillations. In contrast to the synchronisation and the coupling, a correlation does not necessarily mean that one oscillation influences the other. The only connection between both oscillations might be a third one, which is not necessarily known, but causes the dependency between both oscillations.

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2 Plasticity of biological rhythms

Although biological rhythms are very rigid between single periods, they display a remarkable plasticity to react on other periodic (e.g. seasonal) and unpredictable changes; the strength of a zeitgeber is not absolute, but varies with several factors. For once, there are interspecific differences; temperature seems to be more effective in heterothermic species than in homeothermic. Within one habitat, different zeitgebers can be important for different species. Even within one species, individuals react differently to the same zeitgeber. Within one individual, sensitivity changes with life stages, seasons and even daytime. Additionally, the influence of social partners, population size, available space and food, habitat and weather has been discussed.

The strength of the zeitgeber itself might differ. In the worst case, if no zeitgeber is present, this leads to free-running rhythms, but it is possible that only some rhythms are entrained by the zeitgeber, while others are not, resulting in a "partial entrainment". In a natural environment several zeitgebers are experienced by an animal, but usually they are not in conflict with each other and the organism is entrained to the strongest one, usually light. However, in an artificial situation like an enclosure, stable or laboratory, zeitgebers might compete with each other. It is possible that some parameters of the circadian system will entrain to another zeitgeber than the major part of the system. This has been shown for plasma cortisol and urinary cortisone, which can be entrained to periodic feeding in a light:dark (L:D) regime, while body temperature is entrained by light. It is a case of "selective entrainment".

The sensibility of an organism to a zeitgeber changes during the day. Diurnal animals for example show a stronger reaction to a light impulse in the early morning than in the middle of the night. During the "dead zone", a time span during the physiological day, no reaction at all happens to a zeitgeber impulse. In many mammals, there are also ontogenetical changes to zeitgeber strength like the change from diurnal to nocturnal activity in rats.

The most obvious changes in zeitgeber strength are seasonally determined. Depending on latitude, daylength (photoperiod) can change considerably during the year and therefore potential activity times change. As a result, the onset, end or main peaks of activity often drifts with changing daylength, and the actual phase angle between activity times and sunrise/sunset changes too. Some species change from a diurnal to a nocturnal pattern in cold winter or hot summer.

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3 An overview of activity rhythms

3.1 Daily or circadian rhythms

The best studied rhythms are circadian rhythms. In the field, these rhythms will usually display a period length of exactly 24 hours, while under constant conditions the same rhythm will stabilize at a period length between 22 and 28 hours. Not all 24-hour patterns are endogenously controlled circadian rhythms, some are directly triggered by external stimuli (e.g. artificial feeding) and only persist as long as this stimulus is present (see Part II, Chapter 2-1.4). Under field conditions it will be difficult to recognize a daily rhythm as truly endogenous.

The simplest patterns are found in monophasic diurnal or nocturnal species with one activity period per day. However, these animals display rhythmic variations in their activity levels too and even short resting periods during their activity period.

In addition to monophasic species there are species which are active during most part of the day. Some of them have preferred resting times (e.g. few hours before sunrise) and some are called crepuscular, for they have distinct activity patterns during twilight, but in general they have several activity periods distributed over the complete 24 hours.

Another very common pattern is a bigeminus pattern, which is a pattern consisting of two main peaks per day. It is typical for crepuscular animals with one peak at dusk and one at dawn, but it is common even in so-called monophasic species. Several studies have shown that the bigger peak with the more distinct onset is usually at the beginning of the activity period, while a smaller peak precedes the resting period. The second peak is often small and variable in its timing, so it might be obscured in graphs and not mentioned. These peaks are often related to external stimuli like light, temperature or humidity, but studies have demonstrated their persistence even under constant conditions (for review see Aschoff 1966). It has been discussed that the circadian activity rhythm is not generated by one oscillator, but by two (light-on-oscillator and light-off-oscillator). Evidence for this is given in experiments where under constant conditions the activity rhythm splits and activity displays two rhythms with different circadian periodicity and thus peaks at different times.

3.2 Seasonal or circannual rhythms

Few studies have been able to provide continuous activity data from the field. For bigger mammals, the GPS Collar Plus battery is long-lasting enough to study at a period of at least a year, depending on the GPS schedule. To study circannual rhythms, several years of continuous data are necessary, which will be difficult to obtain with today's equipment. Nevertheless, the study of seasonal patterns during one year is a rewarding research topic and repeated collaring of the same animal allows the study of circannual rhythms.

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In most species, there are seasonal changes in behavior and physiology, concerning reproduction, migration or molt. Reasons for this are changes in weather, food supply and predator abundance. The higher the latitude, the bigger are the seasonal changes in the habitat and the need for adaptation. These changes are met with two strategies: (i) migration, (ii) dormancy, hibernation and other physiological adaptations. Both strategies need time for the animal to prepare, so an immediate reaction to a cold or dry spell might not always be enough.

A parameter with reliable changes during the year is photoperiod. It has often been argued that photoperiod is an external control for breeding and indeed changes in photoperiod can change breeding times. However, breeding activity does not follow photoperiod changes immediately. Instead, transients as in circadian zeitgeber shifts have been observed and breeding cycles can only be changed within certain ranges. Circannual rhythms have been found in a variety of species including migration in birds, reproduction in mammals (e.g. sheep and deer) and hibernation in ground squirrels. To study circannual rhythms, several years of continuous data are necessary, which will be difficult to obtain with today's equipment. Nevertheless, the study of seasonal patterns during one year is a rewarding research topic and repeated collaring of the same animal allows the study of circannual rhythms.

An interesting result has been shown in chaffinches (*Fringilla coelebs*) which had been trained to turn the light on or off themselves: they chose photoperiods with a circannual periodicity and related to these, physiological parameter like moult, fat deposits and sexual activity appeared (Dolnik 1974).

The mechanisms controlling the circannual rhythm are not as well understood as for the circadian rhythm. A model has been given with the Bünning-hypothesis (Bünning 1973). The circadian light receptor follows its own circadian rhythm, which results in a variation of the sensitivity for the zeitgeber light in relation to daytime. Depending on the phase angle of the photoperiod, the season is determined and the circannual rhythm is controlled.

In tropical, equatorial areas, photoperiod remains constant, but food supply changes. Here, cyclic rainfall or food supply itself might act as zeitgeber. In migrating species, for which light is the major zeitgeber, this might not be adequate. Instead, circannual rhythms control seasonal physiological parameters.

In many aspects, circannual rhythms are similar to circadian rhythms, but they are not as common. One difference, however, is the higher resistance of circannual rhythms to phase-shifting than circadian rhythms.

3.3 Ultradian and infradian rhythms

In addition to the circa-rhythm there are a variety of rhythms which do not have an external equivalent. These are the ultradian rhythms with a period length of less than 24 hours and the infradian rhythms of more than 24 hours. This group is heterogeneous, in period length as well as in origin and control.

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Feeding rhythms are often ultradian and depend on factors like food quality, ambient temperature and energy requirements and is rather flexible. The control is not fully understood yet and might be primarily by the digestive system, but for feeding rhythms in voles endogenous pacemaker control has been shown (Gerkema and van der Leest 1991). Infradian rhythms are often found in hormone secretion. Good examples are ovarian cycles in multi-oestrus species, which are often linked with other parameters like activity.

Precise timing of ultradian and infradian rhythms might not be important on first thought, but synchrony in feeding activity between conspecifics has the advantages of groups in aspects of predator avoidance. This strategy is successful in the periodic cicada which reproduces synchronously every 13^{th} or 17^{th} year (Daan 1981). Ultradian rhythms also play an important role in group synchronisation in social species.

While the question whether an ultradian or infradian rhythm is endogenously or externally controlled might not be the major topic for field research, it might be helpful for interpreting the results to know if ultradian patterns are truly rhythmic or have rhythmic components, or if they are random patterns. Persistent, predictable rhythms allow predictions of future patterns and deviations from this rhythm might be caused by the animal's physiology (e.g. oestrus, seasonal changes in the digestive system, disease). Random pattern might be more related to non-periodical external short-term events (e.g. weather, changing food availability) and will therefore be more variable.

3.4 Tidal and lunar rhythms

For species living or feeding in intertidal, estuarine and near-shore zones, circatidal cycles of one ocean tide, usually 12 hours 25 minutes, can be important; the midge *Clunio* is able to calculate the exact timing of the lowest ebb tide, which takes place every two weeks (Neumann 1981). Tidal cycles also dependent on the interplay of solar and lunar cycle, as well as weather; e.g. wind affects the amplitude of the tides, clouds obscure moonlight. Thus, these rhythms are very complex and elements of tidal, semilunar, lunar and circadian rhythms can be found in one species.

In nocturnal species, circalunar cycles of 29.5 days are often found in activity. They are most likely exogenously caused, but have been demonstrated in some species under constant conditions too.

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4 Feedback loops

Many physiological parameters (e.g. blood glucose level, body temperature) need to be kept constant; this is achieved by feedback-systems or regulatory circuits (Figure 86). Here, the controlled parameter has to be kept constant. A controller compares a setting value to an actual value within the organism. If they differ from each other due to a disturbance, the correcting variable acts on the controlled system until the controlled conditions return to the setting value. In some cases the setting value is coupled to the circadian or circannual pacemaker and thus varies periodically due to expected external variations (e.g. seasonal variations in body mass). These regulations cause oscillations in the correcting variable as well as small oscillations in the controlled parameter which is to be kept constant; oscillations in the correcting variable are usually earlier, stronger and easier to recognize than those in the controlled parameter.

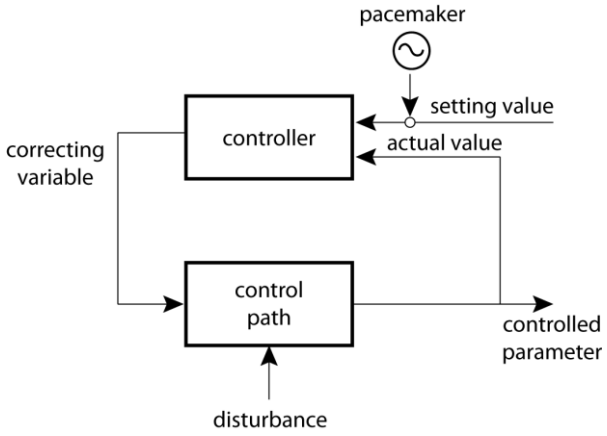

Figure 86: Schema of a feedback loop

Feedback loops and endogenous rhythms interact with each other in a complex hierarchic system, which has been described for the daily pattern of feeding behavior in the Additive Model by Scheibe *et al.* (2001, Figure 87). The framework is given by the circadian rhythm, which is coupled to the circannual rhythm (for details see 3.2). They define the level and the main daily resting and activity times of an organism. Twilight activity rhythms are most likely two separate circadian rhythms and thus part of this framework too. Ultradian rhythms are mostly not controlled by a pacemaker, but depend on external factors like food availability and digestibility and are of high importance in the short time feedback loop regulation of behavior. They are secondary to the circadian rhythms and are coupled to the circadian system in a way that they are enhanced during activity times or suppressed during resting times.The internal control of circadian rhythms.

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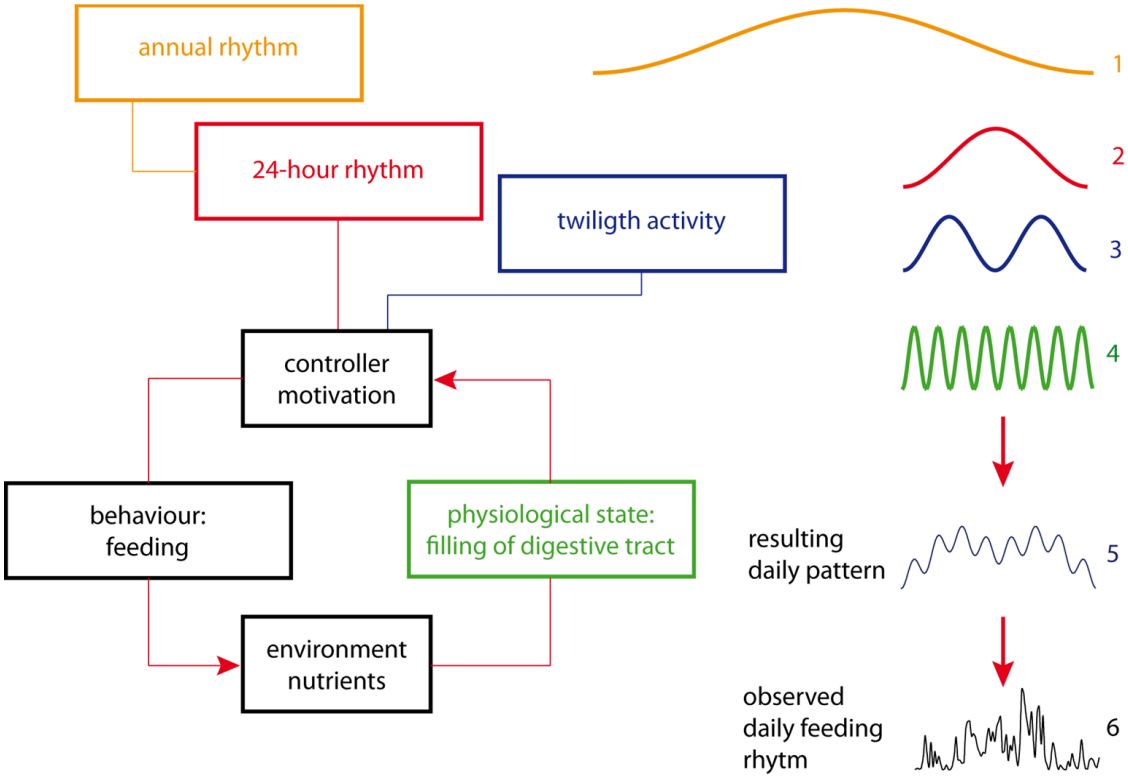

Figure 87: Interaction between endogenously controlled circannual, circadian and twilight-rhythms and the ultradian rhythms as the Additive Model after Scheibe et al. (2001). The circannual (1), the circadian (2), the twilight (3) and the ultradian oscillations (4) are added to a resulting daily feeding pattern (5), which is similar to an observed feeding pattern measured on Przewalski horse in a semireserve (5).

4.1 Neural control

It has been shown that many tissues and even singular cells are able to generate self-sustaining rhythms. In a living organism, these rhythms have to be synchronised by the pacemaker; in vertebrates this is a neural structure within the brain.

In lower vertebrates, this structure is the pineal organ. While the human pineal has no nervous connection to the optic system, in the tuatara (*Sphenodon* spec.), a primitive reptile species from New Zealand, the pineal has a cornea-like structure, a rudimentary lens and a simple retina; it is capable to distinguish light from dark. Additionally it appears to have a secretory function, which reflects the biochemical abilities of the human pineal gland (Ung and Molteno 2004).

In birds, the control mechanisms are more complicated, because different nervous structures have been shown to be involved in circadian control: the pineal organ, the Nucleus suprachiasmaticus (SCN), a part of the hypothalamus situated above the optic chiasma and the retina. There might be peripheral pacemakers too. The importance of the single structures differs significantly between avian species, in some species they interact, in other species only some of these structures are involved in temporal organisation. Input in birds is mainly

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through the retinohypothalamic tract (RHT), connecting retina and SCN, but in every examined bird species there are deep-brain extraretinal photoreceptors.

The mammalian circadian system differs from those other vertebrates: a single primary pacemaker, the SCN, is connected to a single photic sensor, the retina; the RHT is the major or exclusive route for environmental input (Figure 88). The pineal in mammals is not photoreceptive, its rhythmicity is driven by the SCN. If the SCN is removed or isolated, temporal organization of the animal is lost. However, there have been some hints to extra-SCN oscillators, but they have not been located yet.

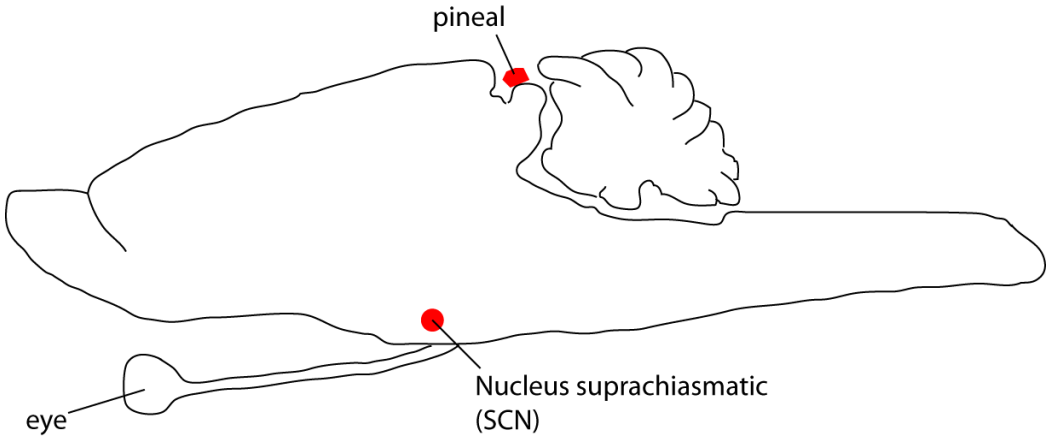

Figure 88: Schema of a mammal brain (median-sagittal)

4.2 Hormonal control

Though the role of the pineal in circadian control might differ between vertebrate classes, a common feature of the pineal is the production of the hormone melatonin. In reptile and avian species, the pineal gland is capable to produce melatonin rhythmically based on its own oscillators, while in mammals, the rhythm of melatonin secretion is coupled to the circadian rhythm of the SCN. Melatonin secretion is suppressed by light, so levels are high during night, and it is known as the “sleeping hormone”. However, melatonin seems to be no direct signal for resting, but a zeitgeber. In diurnal species, the phase angle between melatonin increase and resting period is small, resulting in a correlation between melatonin levels and resting, while in nocturnal species, the increase of melatonin happens at the beginning of their activity period, thus the phase angle is bigger and the reaction of the organism on the melatonin is delayed.

In addition to the circadian rhythm, there is also a seasonal rhythm in melatonin secretion, for in winter, blood levels are higher, especially in higher latitudes with long nights. This way, melatonin is part of the circannual control too.

The mode of action in melatonin is not fully understood, but the favored interpretation is that melatonin acts upon the coupling or phase relationship between several endogenous oscillators. Its secretion is controlled by the pacemaker, whether pineal itself or SCN, but due to back coupling, melatonin influences the pacemaker too.

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4.3 Genetic control

The endogenous rhythms found in behavioral, biochemical and physiological parameters are based on oscillations of specific clock genes. On molecular level, we find transcriptional and translational feedback loops, which have enhancing and inhibiting elements and thus generate circadian rhythms. Similar to the additive model there is a complex, hierarchical system of feedback loops interacting with each other.

Prominent examples of clock genes are *clock* and *per*. Mutations in these genes can result in dramatic changes of circadian rhythms, mostly aberrations in period length, but in some cases even a complete loss of rhythmicity.

It has been shown that the clock genes are not only transcribed in the pacemaker, but that there are self-sustaining rhythms in peripheral tissue too. The expression of single genes is tissue-specific and the coupling of several tissues with each other and with the pacemaker is necessary for a functioning clock system.

5 Disruptions and aberrations of rhythms

If rhythms are as important in an organism's life as discussed, there should be consequences when these rhythms are interrupted. So far, such interruptions have been mainly studied in human medical research. In modern human life, disruptions of biological rhythms and internal desynchronisation have become a considerable problem since they are the consequences of intercontinental travel and shift work. Continuous disruption of circadian rhythms has been associated with a number of adverse mental and physiological conditions. In shift-workers and individuals experiencing frequent jet lag, there is evidence of increased risk of cardio-vascular diseases and cancer, elevated levels of cortisol and deficits in cognitive performance (e.g. Cho *et al.* 2000; Filipski *et al.* 2004; Hansen 2001; Schernhammer *et al.* 2001; Knutsson 1989). In mice, constant jet lag has been shown to decrease life expectancy (Davidson *et al.* 2006). These findings underline the importance of an adequate synchronisation between the various endogenous rhythms within an organism as well as between the organism and its environment.

To understand these findings, it is important to keep in mind that not only many physiological parameters (e.g. metabolic rate, body temperature, heart rate) are rhythmic and linked to the sleep-wake cycle, but also acoustical and optical reaction time, frequency of errors, physical work capacity and cerebral stress bearing capacity, which are important for survival (e.g. Hildebrandt and Strempel 1977). Though the latter has been tested in humans, we can assume that these parameters, which are important for survival, follow endogenous rhythms in animals too. Forced activity or efforts during the physiological resting time would therefore lead to decreases in performance. Since activity patterns are also an adaptation to avoid predators, aberrations from the "normal" pattern would also increase the risk of predation.

With above findings in mind it becomes obvious that in animal management, severe disturbances of endogenous rhythms should be avoided to maintain animal well-being. A

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more direct influence of such disturbances has been shown in hamsters, where heart rate during handling is more elevated at resting times than at activity times, suggesting a higher stress level (Gattermann and Weinandy 1997). Handling and maintenance activities of captive as well as free-ranging animals should therefore take place at appropriate times to keep the disturbance small.

It is obvious that the correct timing of activity is vital for the health and performance of an organism, while aberrations from it can have a severe influence on its fitness. On the other hand, disruptions of endogenous rhythms can be caused by the organism itself. Medical research has shown that there are interruptions in endogenous rhythms linked to several diseases like Alzheimer or depression (Wehr *et al.* 1983). For animals, studies in seminatural and captive conditions have shown changes in activity patterns due to discomfort, stress or disease (Benesch 2007; Berger *et al.* 1998; Berger *et al.* 2003; Scheibe *et al.* 1978; Scheibe *et al.* 1995; Scheibe *et al.* 1999; Schubert 2006). These include restriction and interruptions due to husbandry, influence of hunting, translocations and parasitic infections. Positive events can lead to aberrations in to as has been shown for parturition in mufflon (Langbein *et al.* 1998).

The knowledge of activity patterns, their flexibility and their aberrations can give valuable information on the status of an animal and enable an observer/manager/keeper to restore the conditions necessary for the animal's survival and well-being. Activity patterns can therefore be a useful non-invasive monitoring method. The first step is to obtain a "baseline pattern" or norm-actogram, which illustrates the activity pattern of healthy animals under basically natural conditions (Fleissner 2003; Benesch *et al.* 2005). The "normal" daily pattern can change with age and season and will probably differ between sexes, and there will be differences between single days. These variations have to be taken into account when assessing "aberrations". Therefore, a broad set of data is needed for to establish a norm-actogram for a species. This might be the reason why at present, norm-actograms are only available for few species.

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Chapter 3: Measuring activity

Before you start your study you should give some thought on the way activity is measured with the **GPS Plus Collar**. This chapter will give you an overview on the activity sensors used and how to set up the collar to get valuable data.

1 Characteristics of activity measurement by GPS Plus Collars

GPS Plus Collars measure activity in three axes based on the true acceleration experienced by the collar (Figure 89). Axis X measures acceleration in forward / backward motions, axis Y measures sideward as well as rotary motion and axis Z measures up / down movements using gravitational acceleration.

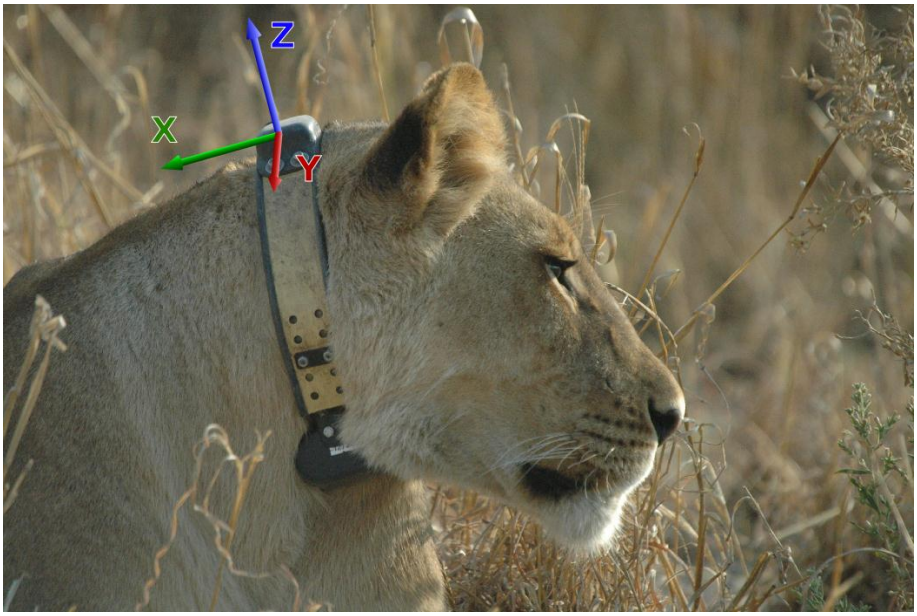

Figure 89: The three axes used for activity measurement by the GPS Plus Collar (Photo: BPCP, Botswana).

Activity is measured four times per second simultaneously on each axis as the difference in acceleration between two consecutive measurements, and is given within a relative range between 0 and 255, characterizing the mean activity / acceleration. Measurements are averaged over the user-selected sampling interval and stored with the associated date, time and temperature.

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2 Basics of sampling intervals

An important question in experimental design is the sampling interval. Fortunately, the storing of activity data in your GPS Plus Collar does not need much energy, so it will not influence the life span of your battery. The memory capacity of the collar is able to store 315128 data points (which for example equals 1094 days in five-minute intervals or 437 days in two-minute intervals). If you choose a sampling interval that will produce a higher number of data points during the batteries lifetime, activity measurements will not be stored after maximum capacity has been reached. Old data will stay in the memory and can be downloaded after retrieval of the collar via a link manager. On demand there is the possibility to download data via the UHF data link and erase the memory before its capacity is depleted (has to be setup when collars are assembled).

The adequate sampling interval depends on your research topic. However, small sample intervals will prevent you from missing important facts about your study of animals' activity pattern. Figure 90 demonstrates how the appearance of a time series changes in relation with sampling interval. With one sample per day, in a rhythmic parameter you will obtain a constant value without any changes during the day (Figure 90a). A sampling interval of 12 hours shows that there are variations during the day, but depending on the actual time of sampling, the results will differ distinctly (Figure 90b). With a sampling interval of six hours, two peaks in the morning and evening appear, but the ultradian peaks are still not visible (Figure 90c). Another problem arises with a sampling interval of five hours, where sampling takes place at different times of day, so the regularity of activity is obscured; days seem to differ from each other and a regularity is not detectable from these data (Figure 90d). These problems can be solved with sampling intervals of four, three and one hours, where activity again is sample at the same time every day (Figure 90e-g). Though the bigger peaks are represented, the ultradian peaks are only visible in the one hour sample. This illustrates the use of small sample sizes, in activity measurements as well as in GPS locations and the incomplete conclusions that can arise from long sampling intervals. For activity measurements, of course, a sample size of less than one hour is advisable, for activity will change with an even shorter frequency. Here, sampling intervals of several minutes are advisable.

Due to the technical parameters of the VECTRONIC system, sampling intervals are based on steps of eight seconds. The smallest sampling interval available in the GPS Plus Collar is 64 seconds. From here, sampling intervals divisible through eight seconds can be chosen depending on the activity measurement mode. Biological rhythm analysis as provided by Activity Pattern is based on the 24-hour-periodicity of activity (for details see Part II, Chapter 4-6 and 4-7). For exact results in time series analysis it is therefore advisable to choose a sampling interval which fits into 24 hours. Based on the technical parameters of the VECTRONIC system, we therefore suggest the intervals of 2, 4 or 10 minutes.

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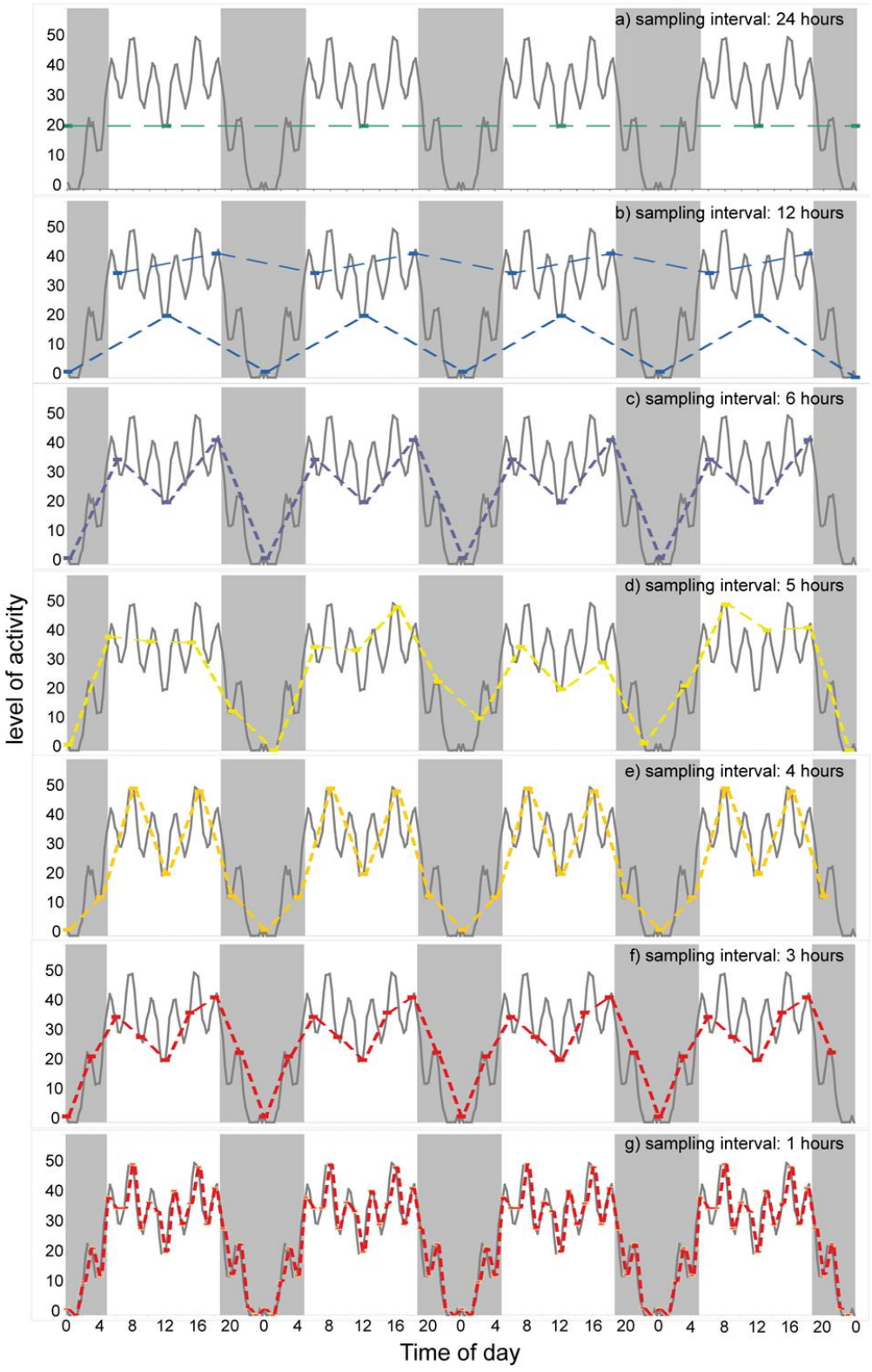
a) sampling interval: 24 hours
b) sampling interval: 12 hours
c) sampling interval: 6 hours
d) sampling interval: 5 hours
e) sampling interval: 4 hours
f) sampling interval: 3 hours
g) sampling interval: 1 hours
level of activity
Time of day

Figure 90: Time series of activity sampled in different intervals. It is obvious that the pattern of activity is not detectable with a long sampling interval and the measured pattern changes with the time of sampling. The smaller the sampling interval, the more precise is the information.

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3 The activity mode

Depending on your research topic you can choose from several measurement modes and sampling interval lengths. Since these settings are not alterable via UHF connection you should give these selections some thought beforehand, especially if you use the collars on wild animals where capture, recapture and handling is difficult.

Depending on your collar type and the firmware version you can select different activity measurement modes. Modes 1-3 measure acceleration on the X and Y axis and store them as two separate values. The modes differ in the length of one sampling interval. In modes 4-7 you can select the sampling interval in steps of eight seconds. The modes differ in the axes and parameters used for measurements.

Mode 1 – 300s Activity Measurement: This mode enables you to store activity data in five minute intervals, but due to the eight second based storing, an activity value is calculated and stored every 296 seconds. The time line is frequently corrected to maintain an effective five minute interval. This results in periodic data gaps, which present problems for further analysis (see Part II, Chapter 3-4 for details on data correction).

Mode 2 – 152s Activity Measurement: This mode measures activity over a sampling interval of 152 seconds.

Mode 3 – 64s Activity Measurement: This mode measures activity over an interval of 64 seconds; it is the smallest storing interval available.

Mode 4: Accumulated activity is measured for a predefined interval; intervals can be chosen in 8 second steps. For time series analysis related to the 24-hour period (i.e. Rhythm analysis and Cosinor), adequate sampling intervals are 2, 4 or 10 minutes, since they are multiples of 8 seconds and integrates of one hour.

Mode 5 – Head Angle / Acceleration Threshold: This mode measures two parameters and stores them in two channels:

1. **Head Angle / Head up**: Gives the ratio of measurements on the X axis within one sampling interval in which the head exceeds a user-defined angle α towards the vertical axis (Figure 91). Maximum value is 255, which equals 100% of the interval. The angle of the head towards the vertical axis is measured using gravity. This mode allows conclusions on a certain behavior, e.g. grazing in grazers (by measuring head angle) or of flying in birds (by measuring the back angle). Comparative preliminary observation is necessary to determine the correct angle in each species and possibly

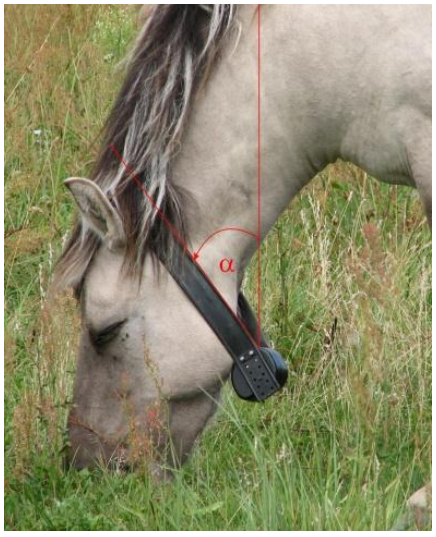

Figure 91: Angle between collar position and vertical axis (Photo: Bianka Schirrmann)

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individual, if necessary on related domestic species, for angle threshold can only be altered via the Link Manager computer connection

2. **Acceleration**: Gives the ratio of combined measurements on all three axes within one sampling interval in which the acceleration exceeds a user-defined threshold (between 0 and 255).

Mode 6 – Acceleration Peak / Acceleration Threshold: In this mode, two parameters are measured:

1. **Acceleration Peak:** The maximum acceleration measurements on all three axes within one sampling interval.
2. **Acceleration Threshold:** The ratio of acceleration measurements on all three axes within one sampling interval below and above a user-defined activity threshold. This mode can be useful to identify activities with high acceleration levels like hunting or activities with low acceleration levels like resting.

Mode 7: Acceleration is measured as forwards-backwards motion (X axis) and head up/down motion (Z axis).

4 Data gaps and interpolation

There are two common causes for data gaps. In older collars, GPS positioning interrupts the activity data storage. Activity is still measured four times per second, but if the hardware is busy at the end of an interval, no dataset is stored. Instead, activity is measured, summed up and averaged over the entire time until the hardware is not busy anymore and the end of the next sampling interval is reached. Thus, the activity value is averaged over two or more sampling intervals instead of one; this value is given for the last sampling interval, the prior intervals are not stored and therefore missing in the ADF file. In new collars, this problem does not arise anymore, activity measurement continues during GPS positioning.

The other cause is related to activity mode 1. In this mode, datasets are labeled every five minutes (300 seconds), but in reality activity is stored every 296 seconds. This results in small deviations between the time labels and the real time in each dataset. To adjust for these deviations, every 74 datasets a data gap of five minutes is inserted.

For some analysis tools, Activity Pattern is able to interpolate data to fill gaps. All data gaps are filled with the activity value of the first sampling interval following the gap. Interpolation of long data gaps however would result in a series of similar activity values, and thus create a false picture of an animal's activity pattern. Interpolation is not available for statistical charts, but for actograms and for data gaps of single sampling intervals in rhythm analysis. If you choose activity mode 1, there will be a periodical interpolation of single datasets, which might influence your results. For rhythm analysis it is therefore advisable to use another activity mode without time correction.

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Data gaps due to GPS positioning or to adjust the time label of mode 1 to the real time are rarely longer than one or two sampling intervals. Longer data gaps hint to other causes. In these cases the continued measurement and averaging of activity cannot be guaranteed; automatic interpolation of such gaps might lead to incorrect results and should be avoided.

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Chapter 4: Data analysis

1 Time series

The GPS Plus Collar collects data as an equidistant time series. Data in time series have a natural order and data close together in time have a higher dependency to each other than other data. To adjust for this temporal order and dependency, time series analysis offers tools for meaningful statistics (e.g. autocorrelation, rhythm analysis) which characterizes the relationships of the data points. Much more, rhythm analysis allows prediction on the future development of the time series.

2 Actogram or activity plot

The first step in analyzing activity data will be to plot the data in an activity plot or actogram.

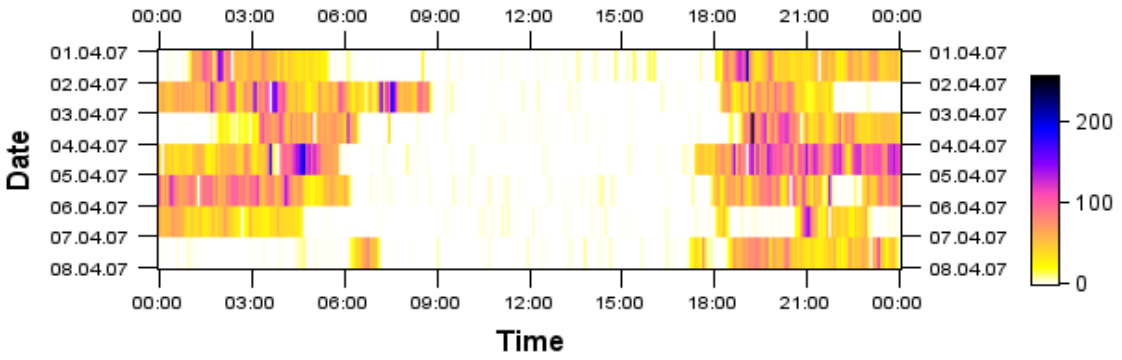

Figure 92: Actogram over eight days. For every sampling interval (in this case every five minutes, resulting in 288 data points per day) the level of activity is indicated by different colours.

In an actogram, the x-axis is the time of day (starting at 00:00 am and ending 24 hours later) and the y-axis is the date (following each other from top till bottom). Every data point is plotted as a box, wherein the level or intensity of activity is given as colour as third dimension. An actogram provides a detailed picture of a certain period in an animal's live and allows you to zoom to time period you are interested in (e.g. a season, rut or a special day or disturbance). If you plot activity over an extended period of time, you obtain a picture of the regularity or variation of daily activity pattern and their seasonal changes.

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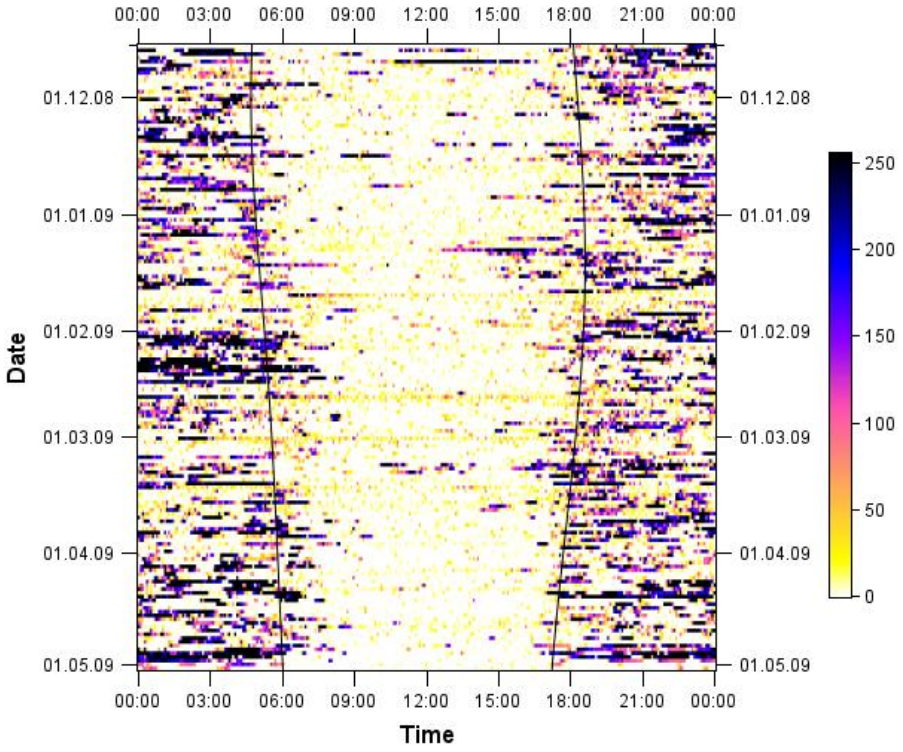

Figure 93: Actogram over about five months for a mostly nocturnal animal. Curved lines indicate sunrise and sunset.

In many cases, a 24-hour-actogram might not be sufficient to display the activity pattern, for the pattern is interrupted at midnight; this is especially irritating in animals which are active during the night. Here, double or even triple plot actograms are useful (Figure 94).

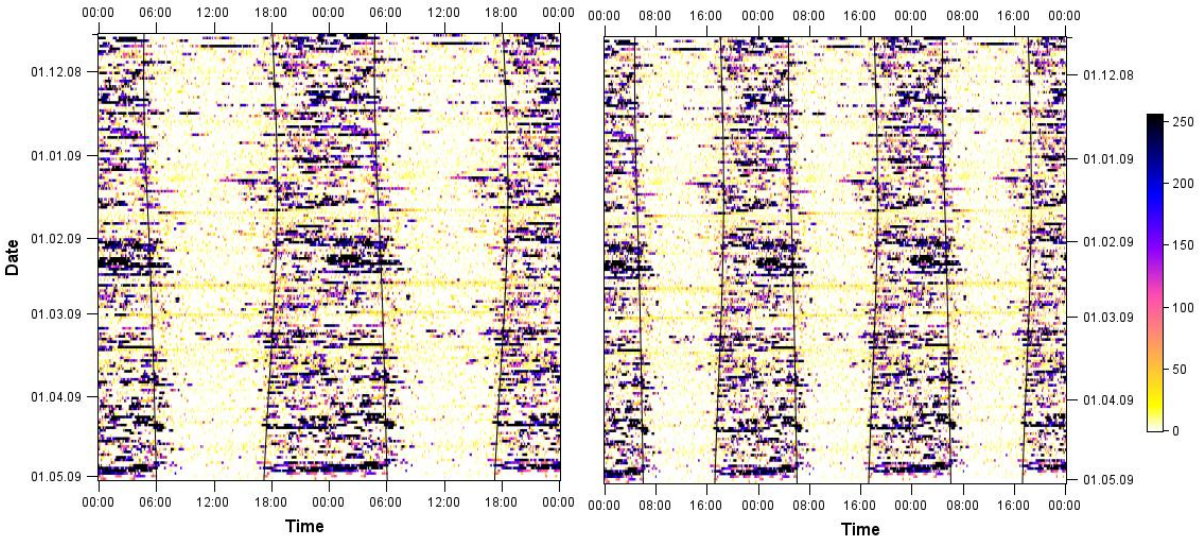

Figure 94: Double (left) and triple (right) plot actogram of the same dataset as in Figure 88. Curved lines indicate sunrise and sunset. The nocturnal pattern is fully plotted and transients over night are visible.

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In a double-plot actogram, the first 48 hours are plotted in one line. The second day of the first line is repeated as first day in the second line and followed by day three (Figure 95).

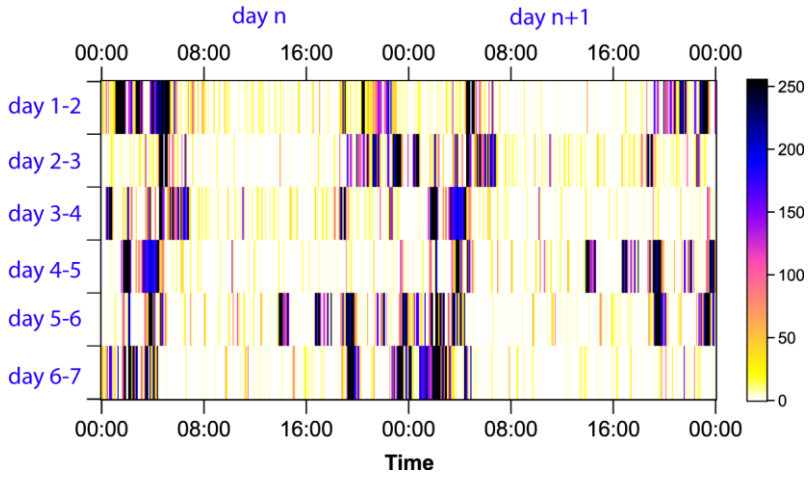

Figure 95: Zoom into double plot actogram over six days.

3 Statistical charts

Charts with mean and median daily activity are well established methods to compare activity between seasons, sexes or populations. This is especially the case for people inexperienced with actograms or to get an overview on activity patterns, e.g. information on times or seasons with especially high or low activity. There are basically two approaches for this method in Activity Pattern:

Variations between single days, months or years (= means by dates, Figure 96A): Mean and median activity for each day or each month give information on changes in activity levels within a certain period of time. They will show either the variation between single days and give hints on the stability of activity budgets, or you can search for differences between months, seasons or notable periods like breeding seasons.

Variations within the course of the day (= means by time, Figure 96B)): The activity pattern over the course of a day as mean or median of activity over several days is often calculated over in intervals of one hour (01:00 – 02:00) or even longer periods of time (e.g. 00:00 – 06:00, 06:00 – 12:00 etc.). They give a general idea of the average distribution of activity during the 24 hours of a day and accentuate peaks, especially for readers not familiar with activity plots.

Two problems have to be kept in mind for interpretation of these form estimates. As already discussed for the sampling interval (Part II, Chapter 3-2), the interval of the data points is important. If means are calculated over one hour or more, the finer pattern of activity is lost (Figure 96). This way you might miss important but short time periods like rut or resting periods.

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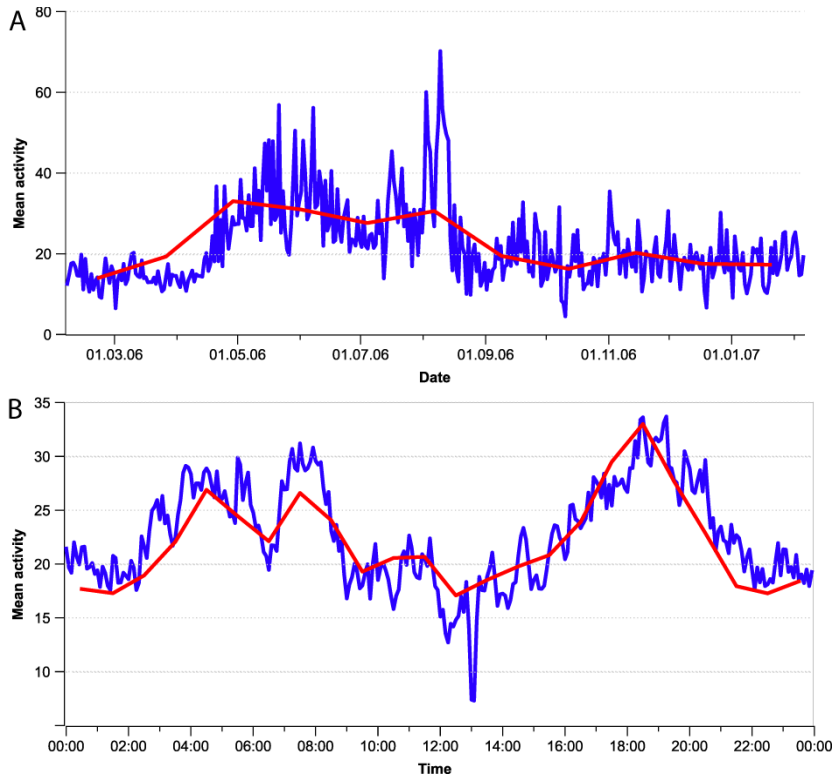

Figure 96: Statistical charts with different sampling interval showing mean values. (A) Statistical by date, blue: sampling interval one day, red: sampling interval one month. The peaks in early August appear only weakly in the monthly sample curve, because of low values in the second half of the month; the peaks in June are leveled out by lower activity values in the same month. (B) Statistical by time, blue: sampling interval 5 min, red: sampling interval 60 min. Short peaks and nadirs disappear.

Care has to be taken in account to the predictability of the peaks calculated by this analysis. Generally, a time series analysis allows predictions on the future pattern by testing the rhythmic components of a time series. Mean and median levels of activity do not give that information. It is tempting to conclude that peaks in the chart are caused by activity that will be displayed at this time every day or year, but this is not always the case. The actogram in Figure 97 shows a daily periods of activity around 9:00 and 16:30 which result in two big peaks in the mean activity chart. The smaller peaks do not correspond to daily activity, but result from activity on some days but resting on others.

The peaks calculated do not necessarily prove the existence of certain rhythms, even if the peaks appear to be rhythmic. A rhythm of for example six or eight hours would result in a peak, but for a rhythm of five hours, the values would level out themselves. The statistical chart will also not show transients (Figure 97, red lines), for they involve small time shifts between days and will therefore not result in peaks. Thus detailed information on activity patterns is lost in statistical charts.

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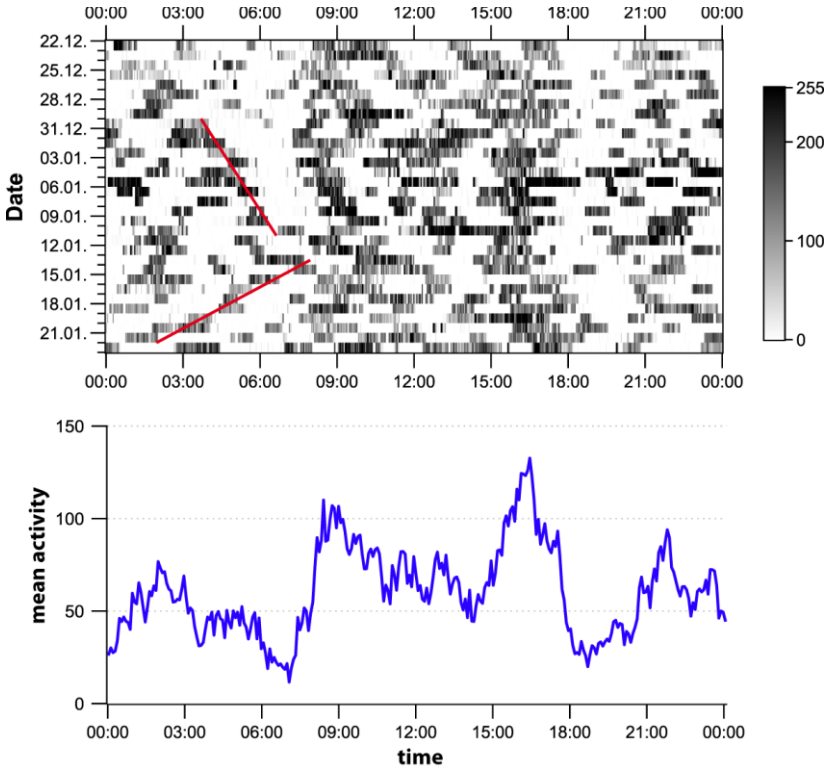

Figure 97: Actogram and associated mean chart by time. While the big peaks are related to a daily period of activity, this is not the case for the medium peaks. In the actogram transients (red lines) are visible which are lost in the mean activity chart.

4 Diurnality Index

The diurnality index or day:night ratio gives the relation of activity during light hours of the day to activity during the dark hours of the same day. Simple calculation of percentage based on sum of activity at day versus sum of activity at night does not account for the different time spans of these two parts of the day. Therefore, actual daylength has to be taken into account.

For **Activity Pattern**, the following equation introduced by Hoogenboom *et al.* (1984) has been used:

(1) $$DiurnalityIndex = \frac{\frac{c_d}{t_d} - \frac{c_n}{t_n}}{\frac{c_d}{t_d} + \frac{c_n}{t_n}}$$

in which c_d resp. c_n is the sum of activity values during the day resp. night and t_d resp. t_n is the number of sample intervals during the day respectively night. This way, the seasonal (or geographical) changes in the relation between daytime and night time are taken into account.

The results of this equation lie between +1 and -1, where +1 is active only during the day, 0 is equally active during night and day and -1 is only active during the night.

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5 Peak Analysis

In addition to the pattern of activity and its total amount or mean for a given time span, the number and duration of activity periods or peaks can give valuable information on the structure of activity. Season, food availability, weather, group size, and reproductive state are just some factors influencing number and duration of activity peaks per day. Herbivores are an interesting group for peak analysis: based on their feeding physiology, browsers (e.g. moose) have generally more and shorter activity bouts per day than grazers (e.g. horses), while in intermediate species (e.g. red deer) there are seasonal changes in food quality and thus in the number and duration of activity peaks.

Since the GPS Plus Collar does not discriminate between active and inactive, but gives a quantitative measure on the intensity of activity, the researcher needs to define a meaningful activity threshold. Due to the characteristics of the collar, this threshold might differ between individuals, but also between body conditions (diameter of neck, thickness of fur). There is no universal algorithm to define this threshold, so mean, median and range of activity values should be considered for every individual tested. The following example illustrates the calculation performed by Activity Pattern.

Input by user:
Peak threshold: 15
Minimal peak length: 10 min

The peak threshold is the mean activity value stored by the collar for one interval. The longer the sampling interval, the higher the probability that short peaks are not accounted for in this analysis. The program checks the time series for intervals in which the activity level is 10 or higher. It then counts the number of successive intervals in which this condition is fulfilled. If the intervals cover 10 minutes or longer, one peak is counted and its duration exceeding the threshold is noted. At the end of each day, the total number of peaks for the last 24 hours (or any other interval selected) is stored and the mean duration over all peaks is calculated. “Peaks” shorter than 10 minutes are rejected for this calculation. Depending on the threshold, number and duration per single peak will differ as is shown in Figure 98. Therefore, the threshold should be tested for every individual and collar before peak analysis is performed to ensure an adequate depiction of the peaks actually measured.

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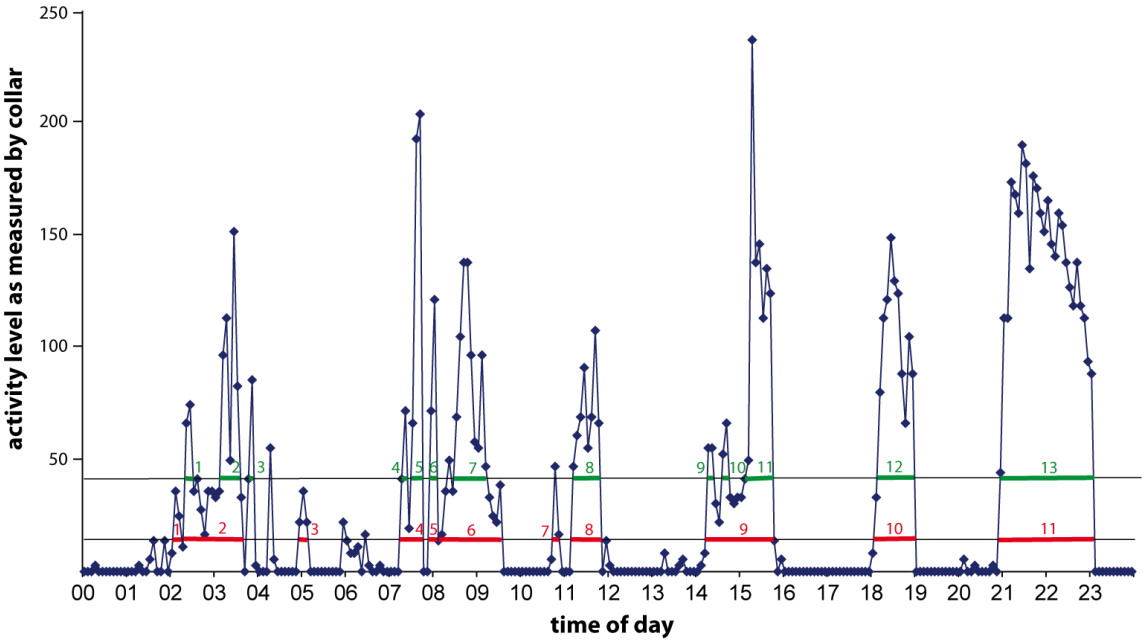

Activity threshold	*Number of peaks in 24 hours*	*Duration of single peak [h]*	*Total activity time in 24 hours [h]*
15	*11*	*0.86*	*9.4*
40	*13*	*0.51*	*6.7*

Figure 98: Exemplary time series with peaks counted for activity threshold 10 (red lines) and activity threshold 30 (green lines). Minimum peak length in both cases 10 minutes.

6 Single Cosinor

Single cosinor is used to justify or not the existence of a given rhythm (e.g. 24 hours) in a time series and calculate its parameters. It also allows to a certain degree to estimate the time the emphasis of activity (or any tested parameter) is located in the time series.

The advantage of cosinor is that it is not sensitive to noise in the data and does not require equally distributed data, so the algorithm can cope with data gaps. With equidistant data as obtain by the GPS Plus Collar, it is also possible to reach relatively large statistical power based on a relatively small sample size.

For cosinor calculation, a cosine wave with a given period length τ (mostly 24 hours or a year of 365 days) is anticipated to the original data. Amplitude, acrophase and mesor of the wave are estimated (Figure 99).

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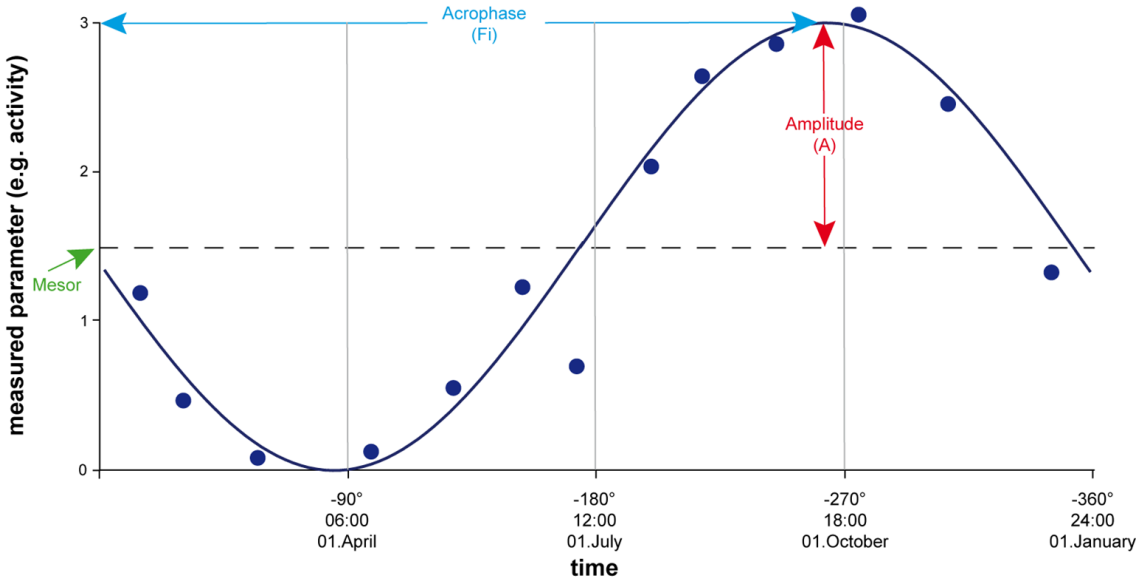

Figure 99: Estimations of acrophase (blue) and amplitude (red) for a period length of 24 hours or 365 days by cosinor calculation

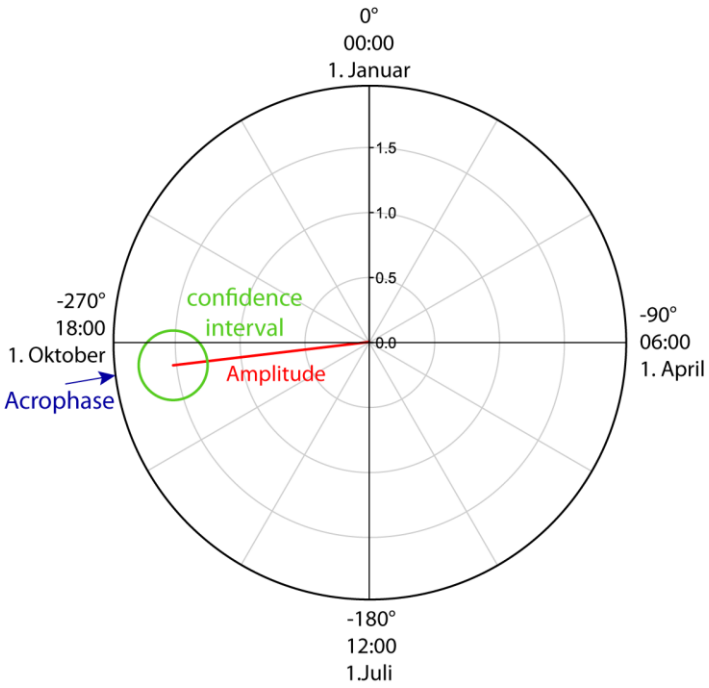

Figure 100: Graphical presentation of Cosinor results. The red line resembles the vector of the Cosinor with the direction showing acrophase (blue), its length indicates the amplitude; the green circle (in some cases an ellipse) indicates the confidence interval. If the radius of confidence interval is bigger than the amplitude, the calculated rhythm is not significant for the selected p-value.

The acrophase describes the phase angle of highest point of the cosine wave in relation to a given start point. It is usually given as vector with the amplitude of the wave (Figure 100). Traditionally it is plotted as a “clock”, where „0°“ or „00:00“ refer to midnight in circadian rhythms and to midnight between 31 December and 01 January in circannual rhythms. The

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complete 360° refer to one full cycle of the rhythm. To plot the acrophase, its degree is given as negative values to plot the vector clockwise, e.g. 04:00 equals -90°. The amplitude represents the difference between the peak of the cosine curve and its mean and thus an estimate for the amount of change of a parameter during one period. The circle shown around the tip of the vector represents the 95% confidence region of acrophase and amplitude. If the circle exceeds the vector, there is no significant rhythm for the given period. The mesor is a rhythm adjusted mean. If a process is known to be rhythmic, but data points are not equidistant or sample size is small, the arithmetic mean may be biased, if for example most of the data points have been sampled around the acrophase. The mesor adjusts for this, and in equidistant data its standard error is usually smaller.

An example for cosinor calculation are the annual variations in the body mass of Przewalski-horses (Scheibe and Streich 2003):

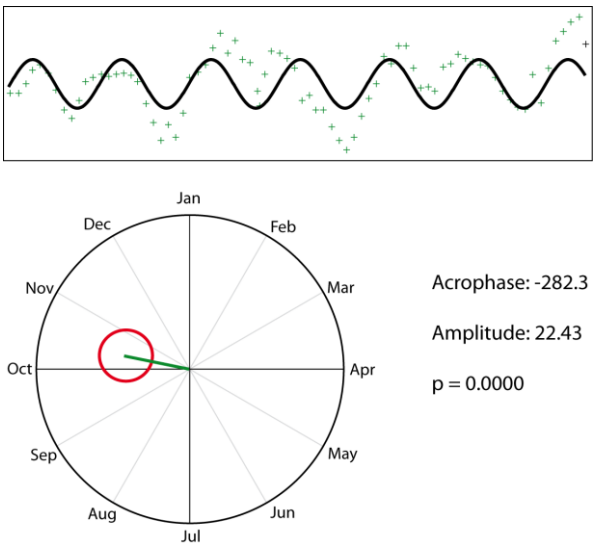

Figure 101: Cosinor calculation for the body mass of Przewalski horses (after Scheibe and Streich 2003,courtesy of authors). A cosine function (black line) with the period length 365 days is fitted in phase and amplitude to the measured time series (green crosses). The acrophase of 282.3 equals the 13th of October, the amplitude was 22.4 kg. The result is significant as indicated by the red circle which is smaller than the amplitude (green vector).

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In this example, the acrophase describes the maximum of body mass in the study animals, but this cannot be achieved with every time series. In daily activity patterns, many species display more than one maximum of activity per day. In crepuscular species for example, maxima are found at dawn and at dusk. If both maxima are similar in height, the acrophase will be found in the centre of both peaks (Figure 102, top). If one of the maxima is bigger than the other, the acrophase will be situated closer to the bigger maximum (Figure 102, center, bottom); Activity at the time of acrophase might actually be low, so the acrophase must not be understood per se as the maximum of activity.

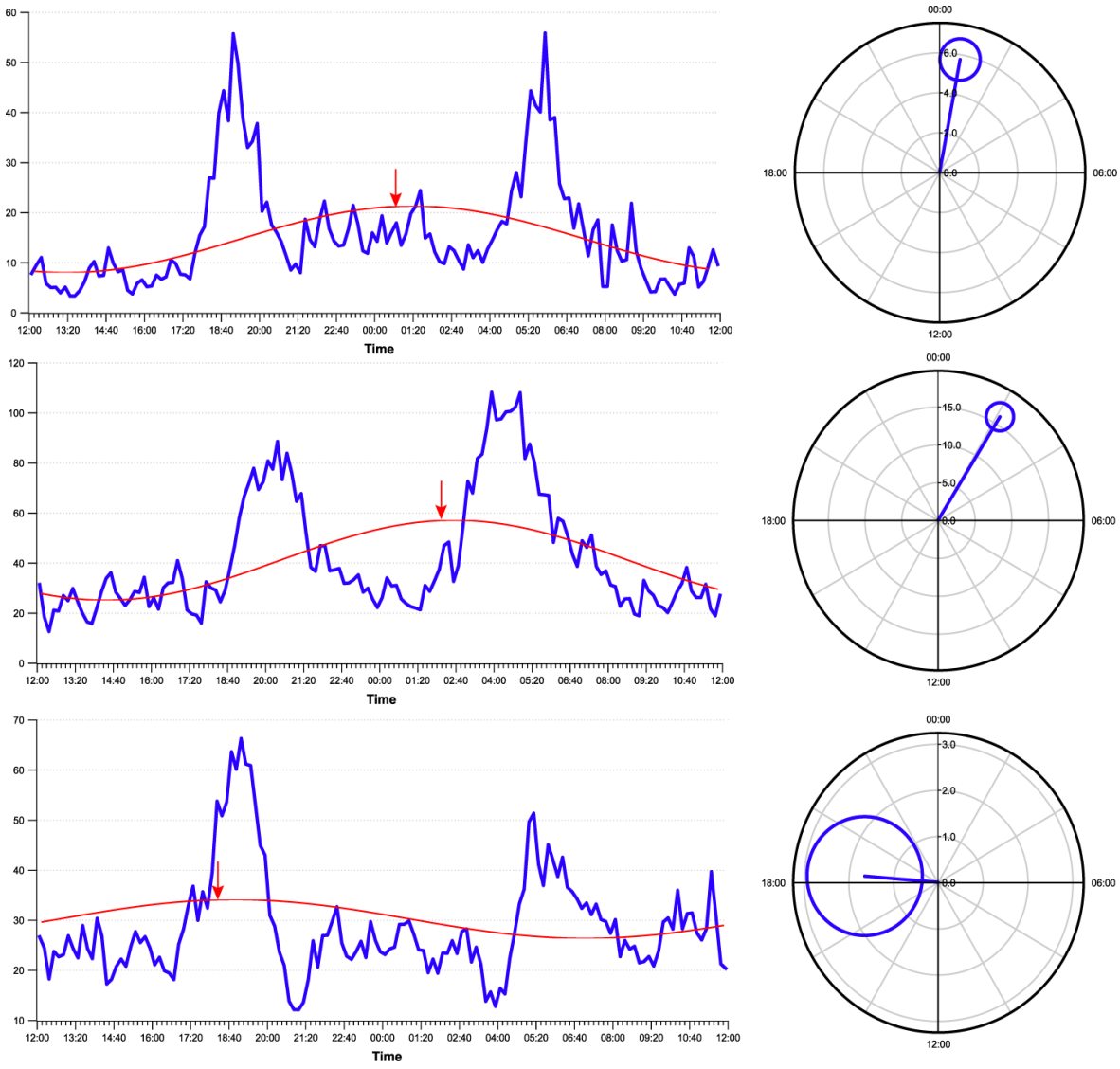

Figure 102: Examples for Single Cosinor results for time series with two maxima. Acrophase differs with the relative size of each maximum and the distribution of the remaining activity.

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7 Rhythm analysis, Harmonic Part (HP) and Degree of Functional Coupling (DFC)

7.1 Aim of rhythm analysis

As discussed in Part II, Chapter 2-4, activity rhythms of animals consist of more oscillations than one; instead, you will in most cases find a combination of circadian, ultradian and infradian rhythms. In Part II, Chapters 1-2 and 2-5 we have discussed the importance of rhythms and their synchronisation to the environment for survival, health and well-being. Activity rhythms have evolved as adaptations to the environment and enable an organism to be active at the times most suited for its physiology or ecology, e.g. regular activity at a certain time of day increases hunting success (Biebach *et al.* 1991). Aberrations from these patterns can result in impairments of fitness, e.g. being active at the wrong time of day increases risk of predation in ground squirrels (DeCoursey *et al.* 2000).

The most important rhythm to synchronise is therefore the circadian rhythm. It is also useful to synchronise rhythms of shorter period length, the ultradian rhythms, with the 24-hour periodicity, so their relation to the circadian rhythm will remain constant to a large extend. This is the case for harmonic ultradian rhythms. They are defined by an integral number relationship with the circadian period, which means that the ultradian period fits to a full amount into the 24 hour period (e.g. 12 hours, 8 hours, 6 hours, 4.8 hours and so on).

The Harmonic Part (HP) and the Degree of Functional Coupling (DFC) have been developed as a measure of such synchronisation (Sinz and Scheibe 1976; Berger *et al.* 2003); they provide an easy-to-handle estimate of synchronisation between an individual and its environment which can easily be compared between several individuals or time spans. The Degree of Functional Coupling will describe this synchronisation with a value between 0 and 1. An individual whose time series consists only of harmonic periods will have a DFC of 1, and is highly synchronised with the 24 hour periodicity of the environment. High DFCs are often found in healthy animals or those which are strongly diurnal or nocturnal, e.g. in the white-tailed sea-eagle (Krone *et al.* 2009). A DFC of 0 results from a time series without any harmonic periods and indicates that the animal is not synchronised with the solar day. A weak power or complete lack of harmonic periods can be caused by stressors (e.g. transport, hunting) or disease, but also by parturition. For this reason, a combination of DFC, harmonic part, mean daily activity and diurnality can be useful to monitor well-being and health in animals and to detect aberrations in activity patterns (or any other rhythmic parameter) at an early stage.

Note: For calculation of DFC and Harmonic Part with **Activity Pattern**, data series without gaps are necessary. Even a data gap of one hour can result in significant differences. Therefore, datasets with gaps of more than one subsequent sampling interval will be rejected for this analysis, which will result in missing results all data groups in which this day is included.

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7.2 Mathematical background

Calculation of Harmonic Part and Degree of Functional Coupling is based on a complex rhythm analysis which includes several steps automatically performed by **Activity Pattern**. To fully understand the relevance of HP and DFC and its limitations, it is necessary to understand the rhythm analysis performed to obtain these results.

The rhythmic components of a measured time series are determined by a Fourier analysis of the related time series. The Fourier analysis is conducted by a Fourier transform, which has originally been developed for technical and physical time series. The standard Fourier transform is usually applied to regular and predictable time series. In general, biological time series show higher variations in period length and a considerable amount of noise. To filter out noise and enhance rhythmic components, the time series is autocorrelated prior to conduction of Fourier transform. The Fourier transform calculates the amplitudes of a number of discrete periods that describe the autocorrelation function, called coefficients. In Activity Pattern, these coefficients are weighted, leveled, and tested for significance. Usually, the squared coefficients are plotted against their period length in a so-called power spectrum. The significant periods are used to calculate Harmonic Part and Degree of Functional Coupling.

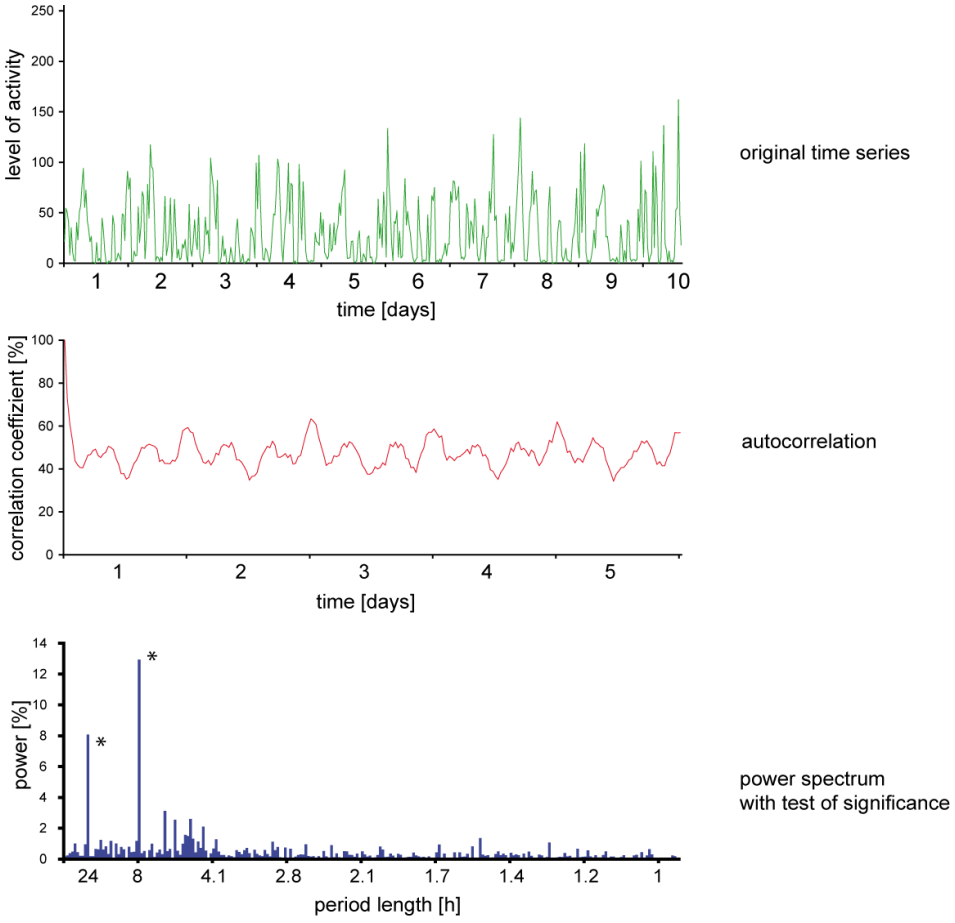

Figure 103: Original time series (period length = 10 days), its autocorrelation and its power spectrum during the time series analysis procedure.

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In the following sections we will describe the procedures and algorithms of these analyses to give a better understanding of the results. Though at present Activity Pattern will only process activity data, these algorithms can be used for any kind of time series, e.g. trajectories, body mass or hormone levels.

7.2.1 Autocorrelation

The first step in rhythm analysis is the autocorrelation, which is used to filter out noise and to enhance the rhythmic components. An example for autocorrelation is shown in Figure 104: a time series of four days (Figure 104a) is duplicated and simultaneous values on both curves are correlated (Figure 104b). Since both curves are identical, correlation is 100%. Then, the duplicated curve is shifted one sampling interval to the right along the time axis and a new correlation is calculated (Figure 104c). This is repeated until only 50% of both curves are overlapping (Figure 104d,e). In regular technical time series, the autocorrelation can be performed until curves do not overlap anymore; this is only possible because technical time series can be assumed to continue unaltered in the future. In biological time series, too many aberrations can occur, so an autocorrelation exceeding the 50% would not be reliable. The resulting functions of autocorrelation (Figure 104f), which are half the length of the original time series, are used for the Fourier transform.

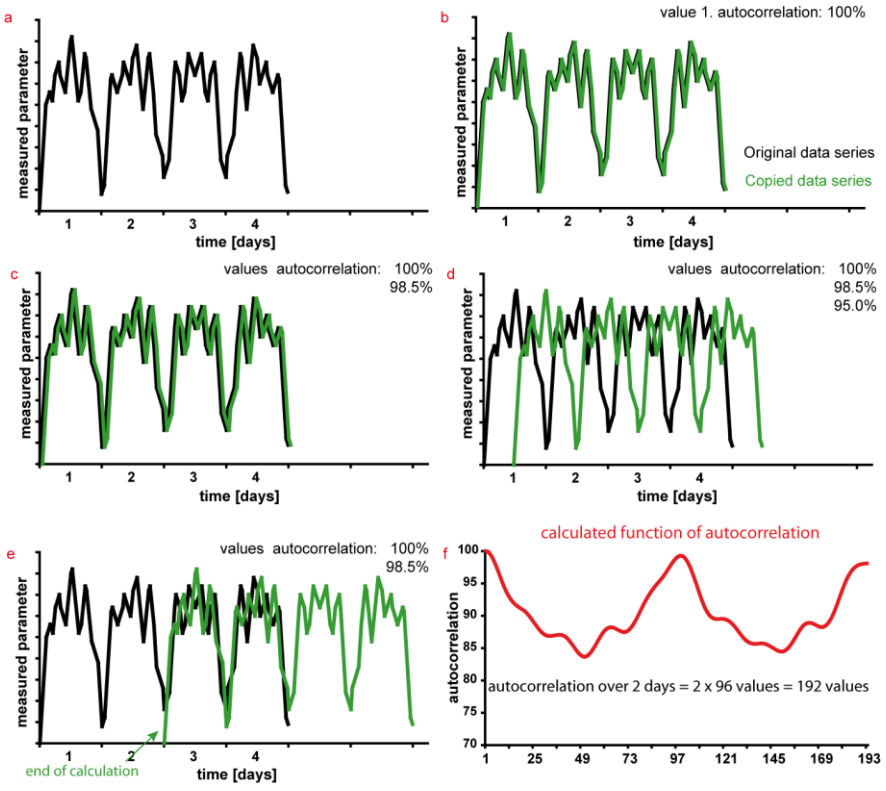

Figure 104: Calculation of autocorrelation for a time series with sampling interval 15 minutes. The original time series (black) (a) is duplicated and correlated with itself (green); the correlation is 100% (b). The duplicated time series is shifted for one sampling interval and again correlated to the original; the correlation is 98.5% (c). Shifting is repeated (d) until only half of the time series are overlapping (e). The autocorrelation coefficients are plotted against the number of sampling intervals for the shift (f).

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The equations used for autocorrelation are the following:

Mean of time series:

(1) $$\bar{x} = \frac{1}{n} \cdot \sum_{i=1}^{n} x_i$$

Function of autocovariance (non-cyclic)

(2) $$c_i = \frac{1}{n} \cdot \sum_{k=1}^{n-i} (x_k - \bar{x}) \cdot (x_{k+i} - \bar{x})$$

$$i = 0,1,\ldots,n-1$$

Function of autocorrelation :

(3) $$r_i = \frac{c_i}{c_0}$$

7.2.2 Fourier transform

As second step, a Fourier transform is conducted the resulting autocorrelation function from the first step. The goal of the Fourier transform is to break down a time series into its rhythmic components and describe the percentage of each component in the original time series. The rhythmic components are called coefficients of the Fourier transform and describe the amplitude of the frequencies. These Fourier frequencies are represented by a number of sine and cosine functions.

Due to the properties of the autocorrelation, which first value is naturally one, only cosine functions are relevant for rhythm analysis. Therefore, the discrete Fourier transform simplifies to a discrete cosine transform, well known in engineering. As the latter one is a special form of Fourier transform, we will continue to speak of Fourier transform, although we only use cosine functions.

The major idea of Fourier analysis is to match the amplitudes of the cosine functions to the autocorrelation function. The number and frequencies for an optimal match depend on the number of data points, so Fourier transforms for time series of different length include different periods as well as a different number of periods. Fourier frequencies are calculated as periods with the following equation:

(1) $$\omega_j = \frac{2\pi j}{n}$$

where $j \in \{1,\ldots,q\}$, with q = n/2 (n even) or q = (n-1)/2 (n odd), n being the number of data points in the sample. Hence the tested periods depend on the lengths of the time series (Part II, Chapter 3-7.2.3); for time series with different lengths, different periods are tested, so they cannot be compared to each other.

In first approximation, for each tested Fourier period the amplitude is calculated which best matches the autocorrelation function (Figure 105). The longest Fourier period tested covers

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the entire length of the autocorrelation function; the shortest Fourier period tested is twice the sampling interval. For better comparison of different time series, the sum of squared amplitudes (power) of all significant periods is defined as 100% and the power of each tested period is given as percentage. For significance test see Part II, Chapter 3-7.2.4.

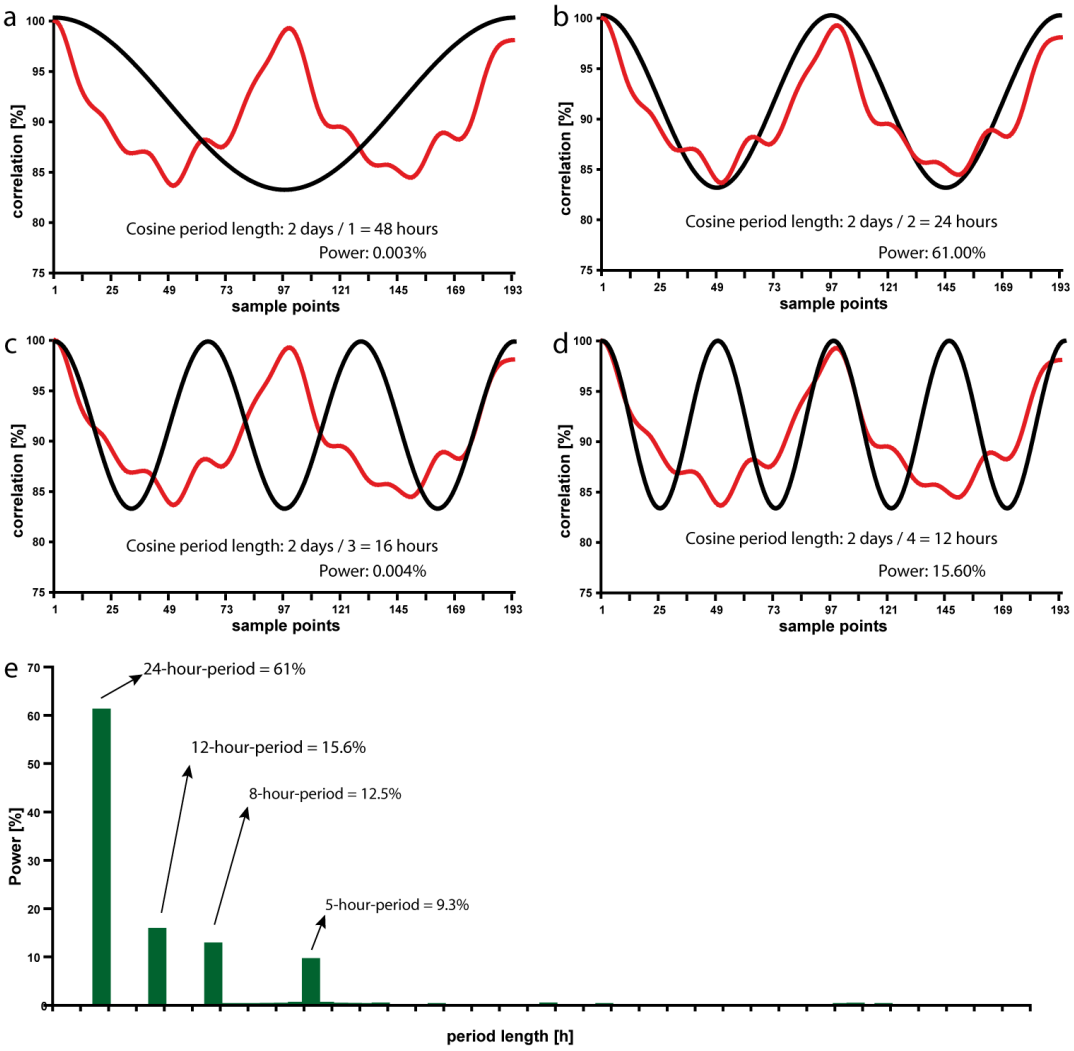

Figure 105: Fourier transform as performed by Activity Pattern. A cosine function which period length equals the length of the autocorrelation function is fitted to the autocorrelation function and its amplitude and power is calculated (a). Then, a cosine function with the period lengths of half the lengths of the autocorrelation function is fitted (b). In the next steps, cosine functions with the period lengths of a third, fourth, fifth, etc. of the autocorrelation function are fitted (c,d). The calculated power of each significant period can be plotted against the period length in a power spectrum (e).

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In **Activity Pattern**, the following discrete Fourier transform is used:

m = n/2 for n even and

m = (n-1)/2 for n odd

$$\lambda = \frac{i}{n}$$

(2) $$I(\lambda_i) = \frac{1}{2}c_0 + \sum_{k=1}^{n-1} c_k \cdot \cos(2\pi\lambda_i k)$$
$$i = 1,2,\ldots,m$$

7.2.3 Basics on the duration of a time series

For understanding and using Fourier analysis it is important to keep in mind that it does not test every period, but only periods which have an integral relationship with the length of the time series, this means that for a time series of 7 days or 168 hours divided by 1,2,3,4, etc. only the periods of 168h, 84h, 56h, 42h, etc. can be tested. The shortest tested period is twice the sampling interval. This implies the following:

1. The tested periods depend on the length of the analysed time series. If you want information on a certain period, you need to choose a time series long enough to test this period. This is especially important if you want to compare two periods, for example 24 and 26 hours. The time series needed to distinguish these two periods is given by $\frac{1}{T} = (\frac{1}{24} - \frac{1}{26})$or T = 312.
2. The tested periods are non-linear, so resolution for short periods is higher than for long periods. To study infradian periods or periods close to the circadian, longer time series are needed than to study ultradian periods.
3. Statistical power depends on the number of times a period is included in a time series. For this reason, short periods have higher statistical power than long ones and the statistical power of a period will increase with the lengths of the time series. To gain an adequate statistical power, a period needs to be included at least seven times, so a time series of at least seven days is needed to test the 24 hour period. Statistical power also depends on the sampling interval. This should be included at least four times in a period.

In addition to these mathematical considerations, the research question is important. Long time series will describe persistent rhythms. Longer periods are plotted in higher resolution and will have higher statistical power than for short time series. However, ultradian periods might not be as consistent over a long time span and might be obliterated or lost in a long time series. This is especially important if HPs and DFCs are calculated to monitor changes in activity. Based on the statistical basics, a time series of seven till ten days has been shown to be most useful to calculate these parameters.

7.2.4 Successive Fisher-test

Not all in the Fourier transform tested periods are significant. To filter out the not significant ones, we use the successive Fisher-test (according to Andel 1984). The power of the tested periods $I(\lambda_i)$ is levelled so that the sum *S* of all power is 100%.

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(1) $$S = \sum_{i=1}^{n} I(\lambda_i) = 100$$

The relative part of each tested period is calculated

(2) $$w = \frac{I(\lambda_i)}{S}$$

and periods are ranked by power level in descending order. For each tested period, p is calculated approximately

(3) $$p = \sum_{j<\frac{1}{w} \wedge j=1}^{m} (-1)^{j-1} \cdot \binom{m}{j} \cdot (1 - j \cdot w)^{m-1}$$

If $p < \alpha$ (α = significance level, e.g. $\alpha = 0.05$) of a tested period, the period is a significant part of the original time series and its power is taken out of the ranking. The sum S of the power of the remaining periods $I(\lambda_1)$ is recalculated and p is calculated for the now highest power $I(\lambda_1)$. This procedure is repeated until a period is reached for which $p \geq \alpha$. This period and the tested periods with lower powers are not significant parts of the original time series, so the procedure stops.

As mentioned before, the statistical power of a period length depends on the number of times this period is included in the time series, for this influences p. If the 24-hour-period is included seven times in a time series, the 12 hour-period is included fourteen times and thus has a higher statistical power. This implies that the probability that a long period length is confirmed as significant part of a time series increases with the length of this time series.

7.2.5 Harmonic Part and Degree of Functional Coupling

The significant periods of the Fourier analysis are used to calculate the Harmonic Part and the Degree of Functional Coupling, two measures for the synchronisation of an organism with the 24 hour solar day. Both are based on the portion of harmonic periods in the original time series. Harmonic periods are defined as periods that are synchronised by an integral number relationship with the circadian zeitgeber (i.e., 24 h divided by 1, 2, 3, etc. gives the harmonic periods 24 h, 12 h, 8 h). The total intensity of all significant harmonic periods in the spectrum is the Harmonic Part HP (respectively Harmonischer Anteil HA in German literature)

(1) $$HP[\%] = SI(harm., sign.)$$

Based on the HP, the Degree of Functional Coupling DFC (respectively *Leistungsbezogener Kopplungsgrad LKG* in German literature) can be calculated

(2) $$DFC = \frac{SI(harm.,sign.)}{SI(total;sign.)}$$

in which *SI*(harm., sign.) is the sum of the power of all significant harmonic periods and *SI*(total, sign.) is the power of all significant periods. While the HP quantifies the intensity of the harmonic periods, the DFC describes the relation between harmonic and non-harmonic

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periods; the higher HP and DFC, the better the synchronisation to the 24-hour periodicity of the environment.

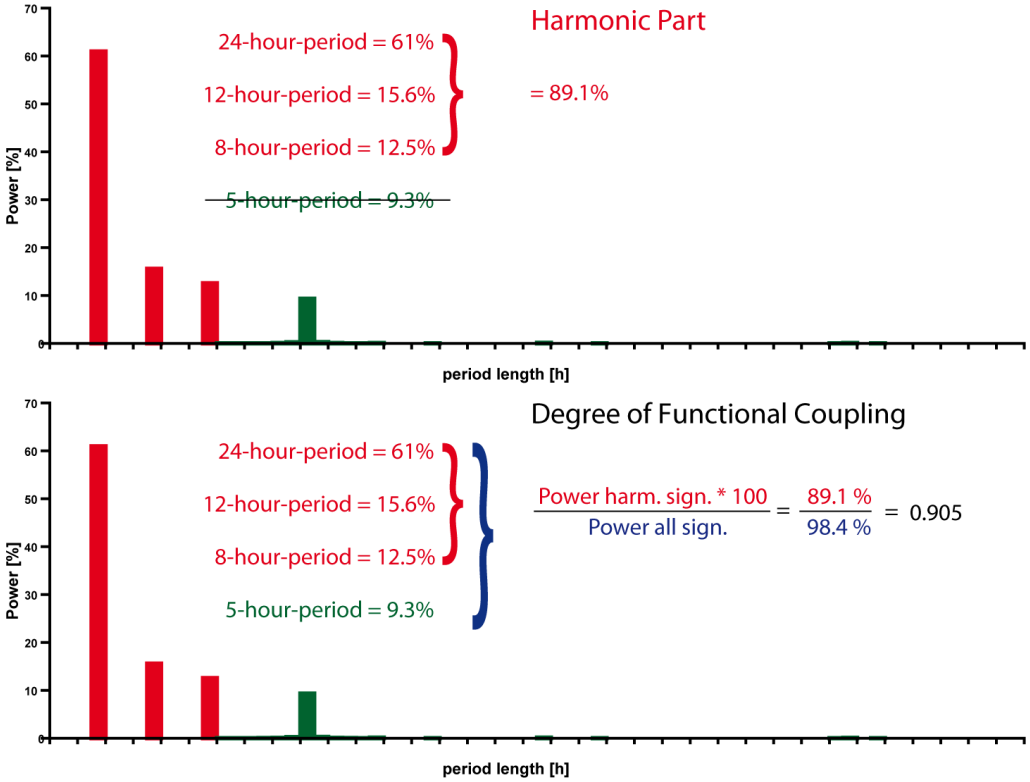

Figure 106: Example for the calculation of Harmonic Part and Degree of Functional Coupling. The Harmonic Part is the sum of the power of all significant harmonic periods. The Degree of Functional Coupling is the relation of the power of all significant harmonic periods and the power all significant (harmonic and non-harmonic) periods.

In **Activity Pattern**, HP and DFC will be calculated for time series of seven or ten days. For better resolution, all datasets will be subdivided into sets of seven or ten consecutive days, with a shift of one day and overlapping six or nine days between successive datasets (that is first dataset covers day 1 to 7, second dataset covers day 2 to 8, third dataset covers day 3 to 9, etc.). This process is equivalent to the procedure of moving average. The resulting HPs and DFCs plotted in the program's output are therefore data not related to one single, but to seven or ten days. Changes in rhythmicity on a certain day will influence all datasets containing this day.

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Glossary

Acrophase – time at which the peak of a rhythm occurs; originally the phase angle of the peak of a cosine wave fitted to the raw data of a rhythm; measured in hours or degrees of circumference in relation to a reference.

actogram – a graphical display of a time series with time of day on the x-axes and date on the y-axes. Successive days are plotted on successive lines, with one cycle per line (single-plot) or several successive days on a line (double-plot, triple-plot, etc.); in the latter case, the first cycle on the line is always the cycle following the first cycle on the line above.

advance – negative phase angle, time between anticipatory behavior and zeitgeber stimulus.

amplitude – difference between the peak and the mean value of a wave.

astronomical dawn/dusk/twilight – defined to begin in the morning and end in the evening when the sun is geometrically 18° below the horizon. At this stage sky illumination by the sun is to faint to be percepted.

bigeminus – diurnal pattern with two peaks, characteristic for crepuscular species.

biological rhythms – recurrent events within a biological system.

chronotope – temporal equivalent to biotope or habitat.

circannual – period length of approximately one year; seasonal cycle.

circadian – period length of approximately 24 hours; from Latin *circa* = approximately; *dian* = day.

circatidal – period length of approximately 12.4 hours, related to the tides.

civil dawn/dusk/twilight – defined begin in the morning and end in the evening when the sun is geometrically 6° below the horizon. At this time illumination is sufficient for humans to clear distinguish their surroundings, but the horizon is still clearly defined and the brightest stars are visible under good weather conditions.

constant conditions – situation without changing external conditions, e.g. constant darkness (DD) or constant temperature; used in chronobiological experiments.

correlation – dependence of two or more random variables or rhythms; it is possible that (i) variable A is dependent from variable B, (ii) variable B is dependent from variable A or (iii) both variables are dependent from an unknown variable C.

coupling – "elastic" link between two or more rhythms; in contrast to synchronisation, coupling allows slow changes in relations between phases and frequencies, but forces them back in a certain relation if they differ to much. In a strong coupling, a common mean rhythm will result ("absolute coordination"), in a weak coupling, a complicated pattern may appear ("relative coordination").

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crepuscular – active mostly at dusk or at dawn or both.

delay – positive phase angle, time between zeitgeber stimulus and reaction of organism.

desynchronisation – loss of a relation in frequency or phase between two or more oscillations within an organism (internal desynchronisation) or between organism and zeitgeber.

diurnal – an event or activity occurring during daytime; in traditional English also understood as daily, which should be avoided in circadian physiology.

endogenous rhythm – rhythm of internal origin; under natural conditions entrained by a zeitgeber, under constant conditions free-running.

entrain – to synchronise a self-sustaining rhythm; done by a zeitgeber.

free-running rhythm – self-sustaining rhythm in the absence of effective zeitgeber or external time cues that may entrain the rhythm.

infradian – period length of more than 24 hours.

frequency – number of events in a defined period of time.

internal desynchronisation – state in which different oscillations within an organism oscillate on different period lengths.

L:D regime – relation of light to dark, in chronobiology written as L:D 10:14 for 10 hours of light plus 14 hours of darkness. See "photoperiod".

masking – direct influence on biological rhythm by an external agent without change of period length.

midday – transit time of the sun through an imaginary line in the sky. Midday is approximately, but rarely exactly 12:00.

nautical dawn/dusk/twilight – defined begin in the morning and end in the evening when the sun is geometrically 12° below the horizon. At this time, the outlines of ground objects might be distinguishable for humans. Most mammals should be able to see properly already.

nocturnal – event or activity occurring during nighttime.

norm-actogram – actogram for a species (or an individual) which displays the activity pattern under "normal", healthy conditions. It takes species-specific variations between days, seasons, sexes and age into account and acts as a baseline to detect aberrations caused by stress, disease, etc.

Nucleus suprachiasmaticus (SCN) – part of the hypothalamus, pacemaker for circadian rhythms in mammals and some avian species.

oscillation – periodic variation of a physiological or logical parameter, usually above and below some mean value.

pacemaker – a functional structure which is able to generate endogenous rhythms and imposes them on other structures.

partial entrainment – steady-state, in which only some of the biological rhythms of an organism are entrained by a zeitgeber, the other are free-running.

period length τ – the time elapsed for one complete oscillation or cycle.

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phase angle φ - predefined point of the oscillation, often the onset of maximum of the measured parameter and can be given in radians, degrees or other fractions of the whole period, e.g. in time units, if period length is given.

phase angle difference $\Delta\varphi$ – time lag between the occurrence of the zeitgeber and the phase angle.

phase shift – single displacement of the phase on the time axis.

photoperiod – relation of light to dark, see L:D regime.

pineal gland – *epiphysis*, endocrine gland that produces melatonin, control organ for biological rhythms in birds an reptiles.

power – square amplitude of a period as calculated by Fourier analysis.

retinohypothalamic tract (RHT) – photic input pathway from the retina to the Nucleus suprachiasmaticus in mammals.

rhythm – periodically recurring event.

selective entrainment – steady-state in which some rhythms of an organism are entrained to one zeitgeber, while other rhythms are entrained by another zeitgeber.

sunrise – instant at which the upper edge of the sun appears above the horizon.

sunset – instant at which the upper edge of the sun disappears above the horizon.

synchronisation – an action that causes two or more rhythms on the same time and/or proceed at the same rate.

time series – values measured in equidistant time intervals as function of time.

transient – temporary oscillation between to steady-state oscillations, usually appears after a phase shift.

ultradian - period length of less than 24 hours.

zeitgeber – periodically appearing external signal that is able to synchronise (entrain) an endogenous rhythm to the environment.

Symbols

τ tau, period length of a biological rhythm

φ phase angle of a biological rhythm

$\Delta\varphi$ phase angle difference

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Index

A

aberration, rhythm · 99, 117
acceleration · 101
  gravitational · 101
  peak · 105
  sensor · 80
  threshold · 104, 105
acrophase · 61, 64, 113, 114, 116, 125
activity · 79, 83, 84, 85, 87, 89, 92, 101
  forced · 99
  induced · 87, 89
  level · 32
  locomotor · 79, 80, 85
  measurement · 101, 102
  mode · 102, 104
  pattern · 80, 81, 82, 83, 90, 99, 100, 107, 108
  peak · 93
    duration · 66, 67, 72, 112
    length, minimal · 112
    minimal duration · 68
    number · 66, 72, 112
    threshold · 68, 112
  plot · 107
  rhythm · 80, 93
  threshold · 32
  time · 66, 71
activity mode · 77
actogram · 31, 107, 125
  colour · 37
  double plot · 108
  triple plot · 108
actual value · 96
adaptability · 83
adaptation · 80, 81, 89, 90
additive model · 96, 97
ADF file · 18, 19, 22, 46, 47, 55, 58, 59, 62, 63, 67, 68, 74
ADF Merge · **74**, **75**, **76**, **77**
advance · 89, 125
age · 82, 92
Alpha values · 47, 59
amplitude · 61, 64, 85, 113, 114, 121, 125
animal management · 99
anticipatory behavior · 87, 90
arithmetic mean · 115
autocorrelation · 57, 59, 119, 120, 121
axes · 34, 35, 36, 48
  bottom · 36, 49
  colour · *35*, 52
  font · 35
  font colour · 35
  font size · 35
  font style · 35
  left · 36, 50
  right · 36
  top · 36
  width · 35, 52

B

background · 39, 52
behavior, anticipatory · 90
bigeminus · 93, 125
biological rhythms · 85, 86, 88, 92, 99, 125
birds · 81, 85, 94, 97, 98
birth · 100
Bünning-hypothesis · 94

C

calendar · 29, 41, 46, 58, 63, 67
cancer · 99
canvas · 33, 48
cardio-vascular diseases · 99
CFG file · 27
channel · 31, 46, 47, 55, 58, 59, 63, 67, 68
chart · 25
  bar · 47, 68
  line · 47, 68
  size · 48
  tab · 18
charts · 24
chronotope · 80, 81, 125
circadian · 125
  clock · 87
  rhythm · 86, 87, 93, 94, 95, 96, 97, 98, 99, 114, 117
circalunar rhythm · 87
circannual rhythm · 86, 93, 94, 96, 98, 114, 125
circatidal rhythm · 87, 95, 125
climate · 82, 83
clock genes · 99
coefficients
  autocorrelation · 119
colour · 29, 47
  actogram · 37
  axes · 35
  background · 32, 39

This design is the property of VECTRONIC Aerospace GmbH. Unauthorized duplication or distribution to a third party is prohibited.

dot · 47
font · 34, 48, 51, 53
frame · 35
line · 47, 55, 58, 59, 68
overlays · 40
palette · 37
vector · 63, 68
conditions
constant · 87, 95, 125
controlled · 96
natural · 87
confidence interval · 46, 53, 58, 59, 61, 64, 114
constant conditions · 87, 95, 125
control
genetic · 99
hormonal · 98
neural · 97
controlled system · 96
controller · 96
coordination · 81
absolute · 91
relative · 91
correcting variable · 96
correlation · 125
cortisol · 81
cosine
curve · 63
function · 121
transform · 120
wave · 61, 114
cosinor · 61, 113, 114, 115
acrophase · 61, 64, 114, 116
amplitude · 61, 64, 114
confidence interval · 61, 64, 114
mesor · 64, 115
period length · 113
vector · 114
count · 46, 53, 58, 59
coupling · 89, 91, 98, 125
crepuscular · 80, 93, 116, 126

D

data
acceleration · 22
format · 36, 50, 51
gap · 105, 117
range · 32, 46, 58, 63, 67
series · 45, 46, 47, 55, 57, 59, 62, 63, 66, 68
tab · 18
database · 20, 21, 29, 31
Database · 19
database connection · 20, 29
dataset · 19, 20, 21, 31, 46, 59
date · 34
range · 35
dawn · 70
day · 69
day grid · 40, 41
dead zone · 92
defaults · 27
Degree of Functional Coupling · 57, 58, 59, 60, 117, 118, 122, 123, 124
delay · 89, 126
dependent data · 107
desynchronisation · 126
internal · 99
deviation
time · 105
DFC · 58, 117
dialog
colour · 29
discomfort · 100
disease · 95, 100, 117
disruption, rhythm · 99
disturbance · 96, 100
diurnal · 80, 81, 86, 88, 92, 93, 126
Diurnality Index · 66, 67, 69, 70, 71, 111
dot
colour · 47, 68
shape · 47, 59, 68
size · 47, 59, 68
double plot actogram · 108
download · 20, 21
dusk · 70

E

Encapsulated PostScript · 26
endogenous rhythm · 87, 88, 96, 100, 126
enhancement · 90, 99
entrainment · 87, 88, 90, 92, 126
partial · 92, 126
selective · 92
environment, artificial · 92
environmental periodicity · 87
EPS · 26
error bars · 46, 58
export · 25
external stimulus · 87, 90, 93

This design is the property of VECTRONIC Aerospace GmbH. Unauthorized duplication or distribution to a third party is prohibited.

F

feedback loop · 96, 99
field conditions · 82
Fisher-test, successive · 122
fitness · 82, 83, 99, 100, 117
flexibility · 83
font · 34
  axes · 35
  colour · 51
  size · 34, 35, 48, 53
  style · 34, 35, 48, 53
food
  availability · 87, 89, 92, 94, 95, 96, 112
  quality · 95
Fourier
  analysis · 118, 122, 123
  coefficients · 118, 120
  frequencies · 120
  period · 120
  transform · 57, 118, 119, 120, 121, 122
frame · 34
  colour · 35
  width · *35*
free-run · 92
free-running
  period · 86
  period length · 86, 88
  rhythm · 86, 87, 126
frequency · 85, 126

G

GPS Plus database · 19, 20, 31
GPS positioning · 80, 83, 105, 106

H

habitat · 80, 82, 83, 92
handling · 100
harmonic
  period · 117, 123, 124
  rhythm · 117
Harmonic Part · 57, 58, 59, 60, 117, 118, 122, 123, 124
head angle · 104
health · 99, 100, 117
hibernation · 94
histogram · 32
hour grid · 40, 41
HP · 117
humidity · 93
hunting · 83, 100
husbandry · 83, 87, 100
hypothalamic-pituitary adrenal axis · 84
hypothalamus · 97

I

infradian rhythm · 86, 94, 95, 126
inhibition · 90, 99
installation · 17
internal
  clock · 82, 87
  desynchronisation · 126
  synchronisation · 87
interpolation · 32, 105
  actogram · 105
  rhythm analysis · 105
  statistical chart · 105
intervals · 69

J

jet lag · 89, 99

L

L
  D regime · 126
label
  axes · 35
labeling · 25
labels · 34, 35, 51
latitude · 42, 68, 92, 94
legend · 44, 52
  width · 44
light · 87, 89, 93, 94
  dark
    frequency · 88
    ratio · 88
line
  colour · 47, 55, 58, 59, 68
  vector · 63
  width · 47, 58, 59, 63, 68
lines · 35, 52
  horizontal · 51
  vertical · 50
local time · 32
locomotor activity · 79, 80, 85
log
  cosinor · 64

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rhythm analysis · 60
statistical chart by date · 53
time budget · 71
longitude · 42, 68
lunar rhythm · 95

M

mammals · 81, 85, 92, 93, 94, 98
margins · 35, 48
masking · 90, 126
maximum value · 51
mean · 45, 46, 53, 54, 58, 59
arithmetic · 86, 115
rhythm adjusted · 85, 115
median · 45, 46, 53, 54, 58, 59
melatonin · 98
memory capacity · 102
mesor · 64, 85, 113, 115
midday · 42, 43, 126
midnight · 42, 43
migration · 82, 94
minimum value · 51
monophasic · 93
moonlight · 95
moult · 94
multi-oscillator system · 91

N

natural conditions · 87
night · 69
nocturnal · 80, 81, 86, 88, 92, 93, 126
norm-actogram · 100, 126
Nucleus suprachiasmaticus · 97, 126

O

oestrus · 81, 95
opacity · 40
optic chiasma · 97
oscillation · 85, 88, 89, 91, 96, 99, 117, 126
oscillator · 93, 98
output options · 25
overlays · 40

P

pacemaker · 87, 90, 91, 95, 96, 97, 98, 99, 126
peripheral · 97
PAL file · 38
palette · 37
parturition · 82, 117
pattern
feeding · 97
peak · 55
analysis · 112
threshold · 68
period · 113
free-running · 86
harmonic · 117, 123, 124
length · 85, 87, 90, 91, 93, 121, 126, 127
free-running · 86, 88
power · 121, 122, 123
periodic noise · 87
phase
angle · 88, 89, 90, 92, 94, 98, 127
angle difference · 127
shift · 89, 94, 127
photoperiod · 83, 92, 94, 127
phylogenetic groups · 81
physiological
activity time · 81
day · 92
resting time · 81, 99
pineal · 97, 98, 127
plasticity · 92
PNG · 26
population size · 92
Portable Network Graphics · 26
power · 121, 127
spectrum · 121
statistical · 122, 123
predation · 81, 83, 90, 94, 95, 99

R

receptor · 87
reproduction · 94
reptiles · 97, 98
resynchronisation · 89
retina · 87, 97, 98
retinohypothalamic tract · 98, 127
RHT · 98
rhythm · 127
aberrations · 99
biological · 88, 99, 125
circadian · 86, 87, 93, 94, 95, 96, 97, 99, 114, 117, 125
circalunar · 87
circannual · 86, 93, 94, 96, 97, 98, 114, 125
circatidal · 87, 95, 125

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disruption · 99
diurnal · 93
endogenous · 87, 88, 96, 100, 126
feeding · 95
free-running · 86, 87, 126
infradian · 86, 94, 95, 126
lunar · 95
seasonal · 86, 93
semilunar · 95
tidal · 95
ultradian · 86, 94, 95, 96, 97, 117, 127
rhythm adjusted mean · 115
rhythm analysis · 57, 59
rut · 82

S

sampling interval · 55, 101, 102, 103, 104, 109, 110, 112, 122
SCN · 97, 98
season · 82, 112
seasonal rhythm · 86, 93
selective entrainment · 127
semilunar rhythm · 95
setting value · 96
settings · 27, 31
sex · 82
shift work · 99
size
chart · 48
dot · 47, 59, 68
font · 48, 51
sleeping hormone · 98
social
partners · 89, 92
zeitgeber · 87
spatial use · 83
standard
deviation · 46, 53
error · 46, 53
statistical chart
by date · 45, 109, 110
by time · 54, 109, 110
stress · 84, 100, 117
stress hormone · 84
sun
lines · 40, 42
position · 69
sunrise · 42, 43, 70, 127
sunset · 42, 43, 70, 127
Suprachiasmatic Nucleus · 98
survival · 86
synchronisation · 99, 117, 127

T

temperature · 22, 81, 86, 87, 92, 93, 95
templates · 27
text angle · 36, 50, 51
thermoregulation · 81
threshold
acceleration · 104, 105
activity · 32, 66
peak · 68
tidal rhythm · 95
time · 34
time budget · 66, 68, 80
time series · 107, 118, 119, 120, 127
duration · 122
time-place learning · 81
trajectories · 80, 119
transform
discrete cosine · 120
discrete Fourier · 120, 122
transient · 89, 94, 110, 127
triple plot actogram · 108
tropics · 94
twilight · 42, 70, 87, 93, 96, 97
astronomical · 42, 125
civil · 42, 125
nautical · 42, 126
TXT file · 54, 61, 65, 72

U

ultradian rhythm · 86, 94, 95, 96, 117, 127
ungulates · 83
UTC
correction · 32
date · 22
time · 22

V

vector
colour · 63
line · 63

W

weather · 83, 90, 92, 94, 95, 112

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well-being · 83, 84, 100, 117
width
  axes · *35*, 52
  frame · 35
  legend · 44
  line · 47, 58, 59, 68
wildlife management · 83
workspace · 22, 23

X

X axis · 51

Y

Y axis · 47, 51, 59, 68

Z

zeitgeber · 87, 88, 89, 91, 92, 94, 98, 123, 127
  effect
    differential · 87
    proportional · 87
  shift · 94
  strength · 89, 92

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